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Role of Fruit Waste and Flavanones-Loaded Silica Nanoparticles in Ameliorating Oxidative Stress and Histological Changes in Rat Brain Induced by Acrylamide

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Abstract

Acrylamide (AA) is a known industrial toxic chemical formed during the heating process of foods contain carbohydrates and proteins. AA produced neurotoxicity characterized by progressive neuronal degeneration. This study was designed to investigate the effects of nanoparticles biosynthesized using fruit waste material and two isolated flavanones on AA-induced neuronal damage in Wistar rats. The enzyme activities of oxidative stress biomarkers included superoxide dismutase (SOD) and catalase were significantly increased. Also, the level of malondialdehyde (MDA) increased while the reduced glutathione (GSH) level decreased following AA treatment. AA administered rats showed increased levels of lipidperoxidative product and total proteincarbonyl content, and hydroperoxide, which were significantly modulated by the supplementation of silica nanoparticles (SNs) of hesperitin (Hst) and naringenin (Nrg). The present data showed amelioration with the activities of enzymes antioxidants and levels of reduced glutathione, which were lowered in AA-induced neurotoxicity in rats. Histological observation in rat's brain architecture represented the protective role of SNs of *Citrus aurantium* albedo extract (CAE), Hst-SNs, and Nrg-SNs in AA-induced neuronal damage. This study provides evidence for the neuroprotective effect of the investigated flavanones on AA-induced neurotoxicity by reducing oxidative stress, up-regulating brain antioxidant status, and diminished brain damage.

1. Introduction

The field of nanotechnology develops novel therapeutic nano-sized materials for biomedical and pharmaceutical applications [1]. Silica nanoparticles (SNs) have gained massive applications in the fields of biology and pharmacology [1]. Some bionanocomposites of plant or biologically active ingredients could be prepared by embedding organic spacers into the silicate network to create nanohybrid structures based on silicates with new properties [2]. The natural products mediated nanoparticles are potential remedy for many neurodegenerative conditions.

Naturally available biological, food, and agricultural waste materials have not been extensively investigated for the synthesis of different types of SNs [3]. Fruits of *Citrus aurantium* leave behind a substantial amount of peels and also a large amount of these wastes were obtained as by-products from food processing industry. The rinds (peels) can present similar or even higher contents of valuable compounds in particular, phenolic secondary metabolites as flavonoids, which impart nutraceutical properties to fruit residues [4]. Strong interest in the potentially quintessential role of flavonoids in human brain health has been steadily growing over the years in the scientific community [5]. The Citrus flavonoids, including the flavanones, hesperetin (Hst) and naringenin (Nrg) have been previously reported as multi-functional agents that exhibit powerful free-radical scavenging, anti-depressant, anticholinergic, and dopaminergic neuron protective activities [6, 7]. Oxidative stress is related to overproduction of reactive oxygen species (ROS) in tissues and it is one of the important general toxicity mechanisms for many toxics materials. Acrylamide (AA) is a known industrial toxic chemical and accounts for one of the major health concerns. It formed during the cook heating process of any protein or carbohydrate-rich food items. Accordingly the general population is highly exposed to AA [8]. AA causes brain damage *via* ROS more than any other organ because of its high lipid content, high oxygen turnover and low mitotic rate [9]. AA produces neurotoxicity characterized by progressive neuronal

degeneration and enhances the production of reactive oxygen species and potentially affects brain. AA substantially increase lipid peroxidation (LPO), decrease the level of reduced glutathione (GSH) and antioxidant enzymes (SOD and CAT) in brain.
Many studies have investigated the biosynthesis of SNs using various plant extracts. However, only a few studies have investigated the synthesis of SNs using food waste materials [3, 10].
The aim of the study to determine the efficacies of the neuroprotective effect of fruit rind albedo extract and the abundant isolated flavanones; Nrg and Hst mediated biosynthesis of silica nanoparticles (CAE-SNs, Hst-SNs, and Nrg-SNs) against oxidative damage and histological changes induced by AA in rat brain.

2. Materials and methods

2.1. Preparation of alcoholic extract of fruit waste materials

The fruits were obtained from the private garden in Banha, Qalyubia governorate, Egypt. All the fruits were of eating quality, and without blemishes, or damage. They were washed thoroughly with double-distilled water and dried with tissue paper. The pericarp region was peeled off from the edible part using a peeler. The white, spongy albedo (nonpigmented portion) was recovered by shaving the flavedo (the pigmented portion) from the peel. The albedo were subsequently cut into small pieces (~10 mm) with a knife, divided into 50 g aliquots, and immersed in 250 ml of ethanol (70%) in 500 ml conical flasks. The mixtures were subsequently heated (40 °C) for 15 minutes with continuous stirring using a magnetic stirrer, after which the aqueous alcoholic extract was cooled to room temperature. The extract was then filtered through Whatman number 1 filter paper, collected into separate sterilized bottles, and kept at 4 °C until further use.

2.2. Chemicals

All chemicals used in the present study were of high analytical grade, products of Sigma (USA), Merck (Germany), BDH (England), Riedel de Hàen (Germany) and Fluka (Switzerland). Donepezil hydrochloride HCl (Aricept®, Pfizer) was used as standard drug.

2.3. Isolation and identification of flavanones

Column chromatography of the methylene chloride fraction from the ethanolic albedo extract of *Citrus aurantium* L. resulted in the isolation of two flavanones;Nrg and Hsp. The isolation was carried according to Rizk et al. [3]. The flavanones were identified on the basis of chromatographic properties and comparison of their ^{1}H- and ^{13}C-NMR data with literature values [11, 12].

2.4. Preparation of the nanomaterial based on tetraethoxyorthosilicate (TEOS).

The sol-gel method was used in the preparation of the nanomaterials through hydrolysis and polycondensation of TEOS as a source of SiO_2 containing HCl as catalyst. The preparation was done according to Haroun et al. [2]. The albedo extract, Nrg and Hsp were completely adsorbed and coated with the silicate particles during the sol-gel process.

2.5. Animals

Ninety male Wister strain albino rats weighing100-120 g were selected for this study. They were obtained from the Animal House, National Research Centre, Egypt. All animals were kept in the controlled environment of air and temperature with the access of water and diet.

2.6. Ethics

Anaesthetic procedures and handling with animals were in compliance with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt and performed since being sure that the animals not suffer at any stage of the experiment (Approval no: 13115).

2.7. Doses and route of administration

Acrylamide (AA) suspended in H_2O and was injected intra-peritoneal in a dose of 50 mg/kg body weight (b.wt.) five times weekly for ten consecutive days [13]. *Citrus aurantium* alcoholic albedo extract-(CAE-), hesperetin- (Hst-) and naringenin (Nrg-) silica nanoparticles (SNs) were administered orally five times weekly for six weeks post-AA induction in a dose 100 mg/kg b.wt. [14]. The same was done for Donepezil HCl, a reference drug in a dose 50mg/kg b.wt. [15].
AA suspended in H_2O and was injected intraperitoneal in a dose of 50 mg/kg body weight five times weekly for ten consecutive days [13]. CAE-SNs, Hst-SNs and Nrg-SNswere administered orally five times weekly for six weeks post-AA induction in a dose 100 mg/kg b.wt. [14]. Donepezil HCl, a reference drug, was administered orally five times weekly for six weeks post-AA treatment in a dose 50 mg/kg b.wt. [15].

2.8. Experimental design

Ninety male Wister strain albino rats will be used in the present study. Animals will be divided randomly into 9 groups of ten rats each. Group1 will be normal, healthy control rats. Groups 2– 4 will be normal healthy rats orally administrated with CAE-SNs, Hst-SNs and Nrg-SNs for six weeks, respectively. Animals of group 5 will be injected intraperitoneal with toxic doses of AA for 10 days and served as intoxicated group. Groups 6–8: will be orally administrated with CAE-SNs, Hst-SNs and Nrg-SNs post AA administration respectively for six weeks. Group 9 will be orally administrated with donepezil HCl as reference drug post-AA administration for six weeks.

2.9. Determination of oxidative stress biomarkers

2.9.1. Estimation of glutathione

Glutathione (GSH) was assayed in brain homogenate according to the method of Moron et al. [16].

The method is based on the development of a relatively stable yellow color when 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) is added to sulfhydryl compounds.

2.9.2. Estimation of malondialdehyde

Malondialdehyde was assayed in brain tissue, according to the method of Buege and Aust. [17]. Malondialdehyde is an unstable compound that decomposed to form a complex series of reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generated malondialdehyde (MDA) which has been used as an indicator of lipid peroxidation process.

2.9.3. Estimation of superoxide dismutase

Superoxide dismutase was assayed according to Nishikimi et al. [18]. Superoxide determination is based on the oxidation of nicotinamide adenine dinucleotide reduced disodium salt (NADH) mediated by superoxide radical through a free radical chain of reactions involving thiol oxidation and univalent O_2 reduction

2.9.4. Estimation of catalase

Catalase enzyme was determined in tissue homogenate by the colorimetric assay method according to Aebi [19]. Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O_2 + O_2$$

In the presence of peroxide (HRP), remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (APP) to form achromophore with a color intensity inversely proportional to the amount of catalase in the original sample:

$$2H_2O_2 + \text{DHBS} + \text{AAP} \xrightarrow{\text{HRP Quinoneimine}} \text{Dye} + 4\,H_2O$$

2.10. Estimation of lipid hydroperoxide

Lipid hydroperoxide was determined in tissue homogenate by the colorimetric assay method according to Nelson and Cox [20].

The NWLSSTM lipid hydroperoxide with ferrous iron to form ferric iron and the subsequent reaction of ferric iron with 3,3′-*bis*[N,N-*bis* (carboxymethyl) aminomethyl]-*O*-cresol sulfonephthalein (xylenol orange) to form a chromagen with measurable absorbance at 560 nm.

2.11. Estimation of protein carbonyl

Protein carbonyl was determined in tissue homogenate by the colorimetric assay method according to Zusterzeel [21]. Cayman's protein carbonyl colorimetric assay kit utilizes the DNPH reaction to measure the protein content in tissue homogenates in a convenient 96-well format. The amount of protein-hydrozone produced is quantified spectrophotometrically at an absorbance between 360-385 nm. The carbonyl content can then be standardized to protein concentration.

2.12. Histopathological analysis

Brain slices were fixed in 10% formaldehyde and embedded in paraffin. Sections of 5 mm thickness were stained with hematoxylin and eosin (H and E) then examined under a light microscope for the determination of pathological changes according to Hirsch et al. [22].

3. Results and discussion

3.1. The antioxidant effect of CAE-SNs, Hst-SNs and Nrg-SNs on glutathione and malondialdehyde levels in brain tissue of acrylamide intoxicated rats

CAE-SNs, Hst-SNs and Nrg-SNs administered to healthy rats showed a significant increase in the concentration of glutathione while, an insignificant decrease in MDA level as compared to a normal control group. However, Acrylamide intoxicated rats showed a significant decrease in glutathione level while, significant increase in malondialdehyde by 47.11 and 111.50% respectively compared with normal healthy rats. In comparison with acrylamide intoxicated rats, treatment with CAE-SNs, Hst-SNs and Nrg-SNs as well as donepezil HCl showed significant increase in glutathione level in rats treated with CAE-SNs, Hst-SNs and Nrg-SNs and donepezil HCl. Whereas, significant decrease in malondialdehyde level was detected post treatment of intoxicated rats with CAE-SNs, Hst-SNs and Nrg-SNs well as donepezil HCl with different percentages of amelioration (Table 1).

Table 1: Antioxidant effect of CAE-SNs, Hst-SNs and Nrg-SNs on glutathione and malondialdehyde levels in brain tissue of the different experimental groups

Groups / Parameters	Control	Controls -Treated			Acrylamide	Acrylamide-Treated			
		CAE-SNs	Hst-SNs	Nrg-SNs		CAE-SNs	Hst-SNs	Nrg-SNs	Donepezil HCl
GSH									
Mean± SD	108.75±8.53^{f}	207.50±17.07^{cd}	257.50±25.00^{b}	155.00±12.90^{c}	57.50±25.00^{g}	262.50±15.00^{a}	250.00±18.25^{ab}	228.00±9.09^{bc}	187.50±17.07^{d}
%^{a}	-	90.80	136.81	42.50	47.11	141.42	129.90	109.72	72.42
%^{b}	-	-	-	-	-	356.52	334.78	296.52	226.09
%^{c}	-	-	-	-	-	188.50	177.00	156.81	119.51
MDA									
Mean± SD	8.92±0.33^{bcde}	10.72±0.71^{b}	10.22±0.75^{bc}	8.37±0.45^{cde}	18.87±1.71^{a}	7.90±0.29^{de}	7.72±0.25^{de}	7.45±0.50^{e}	9.67±0.56^{bcd}
%^{a}	-	20.61	14.60	6.21	111.50	11.41	13.50	16.50	8.40
%^{b}	-	-	-	-	-	58.13	59.09	60.52	48.75
%^{c}	-	-	-	-	-	123.00	125.00	128.00	103.11

%^{a}: % change as compared to normal control rats, %^{b}: % change as compared to acrylamide intoxicated rats, %^{c}: % of improvement. CAE-SNs:*Citrus aurantium* albedo extract silica nanoparticle, Hst-SNs: Hesperetin-silica nanoparticle, Nrg-SNs: Naringenin-silica nanoparticle. Data are expressed as means ± SD of ten rats in each group. Data are expressed as μg.mg^{-1} for glutathione and malondialdehyde. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version, 8), where unshared letters between groups are significant value at $P \leq 0.05$.

3.2. *The antioxidant effect of CAE-SNs, Hst-SNs and Nrg-SNs on superoxide dismutase and catalase activities in brain tissue of acrylamide intoxicated rats*

Nanoparticles of CAE-SNs, Hst-SNs and Nrg-SNs administered to healthy rats showed a significant increase in superoxide dismutase activity. Catalase enzyme activity showed an insignificant change in its activity compared with untreated controls one. However, naringenin exhibited significant increase in catalase activity reached to 44.81%. On the other hand, AA intoxicated rats showed significant decrease in SOD and catalase enzyme activities by 45.21 and 80.50%, respectively, as compared to normal healthy rats.Comparing with AA group, significant increase in SOD and catalase enzyme activities was observed for CAE-SNs, Hst-SNs and Nrg-SNs as well as donepezil HCl with different percentages of amelioration (Table 2).

3.3. Effect of CAE-SNs, Hst-SNs and Nrg-SNs on lipid hydroperoxide and protein carbonyl levels

Nanoparticles of CAE-SNs, Hst-SNs and Nrg-SNs administered to healthy rats showed insignificant decrease in lipid hydroperoxideand protein carbonyl levels as compared to normal control rats.

Compared to normal healthy rats, AA intoxicated rats showed a significant increase in lipid hydroperoxide and protein carbonyl levels by 108.30 and 147.90%, respectively.

However, rats treated with CAE-SNs, Hst-SNs and Nrg-SNs and donepezil HCl post AA treatment demonstrated significant decrease in lipid hydroperoxide and protein carbonyl levels for CAE-SNs, Hst-SNs and Nrg-SNs and donepezil HCl comparing to AA-intoxicated rats with different ameliorative percentages (Table 3).

4. Discussion

One of the confirmed neurotoxicant is acrylamide (AA). In commonly consumed human aliments, AA can be found as a result of food processing or cookingin trace amount.The present investigation was designed to evaluate the protective effects of silica nanoparticles of fruit wasteand abundant flavanones against AA induced adverse effects in an experimental rat's model.

Table 2: Antioxidant effect of CAE-SNs, Hst-SNs and Nrg-SNs on superoxide dismutase (SOD) and catalase (CAT) activities in brain tissue of the different experimental groups

Groups / Parameters	Control	Controls -Treated			Acrylamide	Acrylamide-Treated			
		CAE-SNs	Hst-SNs	Nrg-SNs		CAE-SNs	Hst-SNs	Nrg-SNs	Donepezil HCl
SOD									
Mean± SD	57.25±7.32^{c}	89.02±7.25^{ab}	73.15±6.66^{b}	91.80±7.68^{a}	31.35±2.26^{d}	88.47±6.81^{ab}	63.45±4.35^{c}	73.42±5.81^{b}	96.87±6.55^{a}
%^{a}		55.50	27.81	60.30	45.21	54.41	10.82	28.22	69.21
%^{b}	-	-	-	-	-	182.20	102.39	134.19	209.00
%^{c}	-	-	-	-	-	100.61	56.10	73.50	114.40
CAT									
Mean± SD	0.87±0.25^{b}	0.84±0.09^{bc}	0.82±0.14^{b}	1.29±0.22^{a}	0.17±0.02^{d}	0.70±0.15^{bc}	0.90±0.21^{b}	1.58±0.31^{a}	0.53±0.20^{c}
%^{a}		3.45	5.72	44.81	80.50	19.52	3.45	81.60	39.11
%^{b}	-	-	-	-	-	311.76	429.41	829.41	211.76
%^{c}	-	-	-	-	-	60.90	83.91	162.11	41.40

%^{a}: % change as compared to normal control rats, %^{b}: % change as compared to acrylamide intoxicated rats, %^{c}: % of improvement, CAE-SNs: *Citrus aurantium* albedo extract silica nanoparticle, Hst-SNs: Hesperetin-silica nanoparticle, Nrg-SNs: Naringenin-silica nanoparticle, Data are expressed as means ± SD of ten rats in each group. Data are expressed as μmol.mg^{-1} protein for SOD and unit/g tissue of brain for catalase enzyme. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version8), where unshared letters between groups are significance value at $P \leq 0.05$.

Table 3: Effect of CAE-SNs, Hst-SNs and Nrg-SNs on lipid hydroperoxide and protein carbonyl levels in the different experimental groups

Groups / Parameters	Control	Controls -Treated			Acrylamide	Acrylamide-Treated			
		CAE-SNs	Hst-SNs	Nrg-SNs		AE-SNs	Hst-SNs	Nrg-SNs	DonepezilHCl
Lipid hydroperoxide Mean± SD	451.75±10.96^{f}	438.77±10.53^{f}	429.01±10.10^{f}	443.86±12.24^{f}	941.20±12.88^{a}	605.40±13.49^{e}	648.66±11.52^{c}	636.51±12.31^{d}	685.74±13.04^{b}
%^{a}		2.90	5.00	1.71	108.30	34.00	43.60	40.91	50.80
%^{b}	-	-	-	-	-	35.68	31.08	32.37	27.14
%^{c}	-	-	-	-	-	74.31	64.82	67.41	56.51
Protein carbonyl Mean± SD	3.32±0.63^{e}	3.13±0.53^{e}	3.06±0.71^{e}	3.23±0.82^{e}	8.23±0.90^{a}	5.62±0.37^{b}	5.20±0.68^{c}	5.04±0.99^{d}	4.99±0.66^{d}
%^{a}		5.72	7.80	2.71	147.90	69.30	56.61	50.31	50.30
%^{b}	-	-	-	-	-	31.71	36.82	37.76	39.37
%^{c}	-	-	-	-	-	78.62	91.30	96.10	97.62

%^{a}: % change as compared to normal control rats, %^{b}: % change as compared to acrylamide intoxicated rats, %^{c}: % of improvement. CAE-SNs: *Citrus aurantium* albedo extract silica nanoparticle, Hst-SNs: Hesperetin-silica nanoparticle, Nrg-SNs: Naringenin-silica nanoparticle. Data are means ± SD of ten rats in each group. Data are expressed as µg.mg^{-1} protein for lipid hydroperoxide and nmol.mg^{-1}protein for protein carbonyl. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version,8), where unshared letters between groups are significance value at $P \leq 0.05$.

4.1. Antioxidant effect of CAE-SNs, Hst-SNs and Nrg-SNs on antioxidant enzymes and oxidative stress biomarkers in brain tissue of AA-induced rats.

Endogenous antioxidants, including such non-enzymatic scavengers as glutathione (GSH) and such antioxidant enzymes as superoxide dismutase (SOD), and catalase (CAT), are the first lines of defence against oxidative stress and act by scavenging potentially damaging free radical moieties [23].

In the current study, there is a significant decrease in the antioxidant enzyme activities in the brain of AA-intoxicated group with an elevation of oxidative stress biomarkers. This neurotoxicity exhibited by AA may be a result of lipid peroxidation [24] or increase in brain thiobarbituric acid reactive substances followed by membranes damage and alterations in receptor functions [25].

Further, the present data showed the lipid peroxidation was increased while the activities of some antioxidant enzymes in brain tissue of the AA-intoxicated rats were decreased. These antioxidant enzymes involved in

the ROS detoxification, such as SOD, catalase as well as GSH were decreased compared with control displaying the pro-oxidant effect of AA. The reduced synthesis of the enzyme proteins due to high intracellular concentrations of AA may be related to the inhibition the antioxidant enzyme activity carried out in the current study [25].

The reduction in GSH in brain tissue in the present results may be well correlated with the elevation of H_2O_2 cytotoxicity in endothelial cells as a consequence of inhibition of GSH reductase [26]. The significant decrease in the activity of brain SOD in the current study might be also attributed to the excess production of ROS [27] as AA causes brain damage *via* ROS more than any other organ [9].

There is growing interest in the potential beneficial effects of flavonoids in diseased brain. Citrus extract and the two flavanones abundantly found in orange; Hst and Nrg were reported to play important role in plant defence. The administration of the flavanone hesperidin (Hsp), well known antioxidant and anti-inflammatory agent, has offered protection against gamma irradiation-induced oxidative stress in rats [6, 28]. Therefore, it was believed that these selected nanoparticles of hesperitin (Hst-SNs) are considered as a powerful radical scavenger that promote cellular antioxidant defence systems against oxidative stress-induced by toxic agents such as acrylamide [6, 29].

A recent study showed that polyphenolic compounds as flavonoids and anthraquinones had the potential to be used as neuroprotective drugs for traumatic brain injury. They have strong oxidation-resisting characteristic. Polyphenol as rhein was absorbed in the brain tissues of the controlled cortical impact rats. This compound elevated the SOD, CAT activities, GSH level, and diminished the MDA [30].

Hst is one of the ideal natural candidates in the treatment of different central nervous system disorders as it has the ability to cross the blood brain barrier [31].

4.2. Effect of CAE-SNs, Hst-SNs and Nrg-SNs on lipid hydroperoxide and protein carbonyl biomarkers in brain tissue of AA induced rats

In addition the present results clearly demonstrated significant elevation in lipid hydroperoxide and protein carbonyl contents in AA-intoxicated rats. AA is a neurotoxic enhances the production of ROS and potentially affects brain and produces progressive neuronal degeneration. In the current study, the levels of lipidperoxidative product and total proteincarbonyl content, and hydroperoxidewere increased in AA-administered rats. These effects were significantly modulated by the supplementation ofthe two investigated flavanonessilica nanoparticles of hesperitin (Hst- SNs) and naringenin (Nrg- SNs). Citrus flavanonesNrg and Hst are potent antioxidants that may contribute to protecting the striatum from oxidative/nitrosative insults caused by neurotoxin through their antioxidant activity [3, 32]. Flavanoneswere reported to protect neurons against oxidative insults *via* the modulation of neuronal apoptotic machinery [33].

Lysis of cell and membrane damage may be related to the lipid peroxidation induced by free radicals [34]. The neurodegeneration leading to brain tissue damage of AA-intoxicated rats showed an increase in lipid hydroperoxides. On the other hand, histopathological examination of the current study demonstrated that cerebral cortex of AA-intoxicated rats showing pyknosis and necrosis of neurons as well as focal cerebral hemorrhage. While the hippocampus of AA-intoxicated rats showed pyknosis and necrosis of pyramidal cells with neuronophagia of some necrotic cells. In concomitant with the present findings Aly et al. [26] and Sumathi et al. [25] revealed that AA administration causes severe changes in brain as necrosis and neuronal degeneration. This neurotoxin agent was reported to exhibit histopathological lesions in the cerebral cortex as gliosis, odema and cytoplasmic vacuolization hemorrhage [35]. The capability of Hst of activating extracellular signal-regulated kinase 1 and 2 (ERK1/2) signaling in cortical neurons and finally increasing the expression of a number of neurotrophins was reported [33, 36]. The protective potential of Hst in fibroblasts may be mediated both by intracellular scavenging of peroxynitrite and by modulation of fibroblast signalling [37]. The Hsp was reported to exert a protective effect by abrogating oxidative stress, endothelial dysfunction and neurotoxicity [38]. This compound significantly reduced endothelial dysfunction along with the restoration of histological aberrations by increased serum nitrite and vascular nitric oxide bioavailability.Therefore, it is thought that the therapeutic effects of the three selected nanoparticles (CAE-SNs, Hst-SNs and Nrg-SNs) against histopathological changes and lipid peroxidation induced by AA may be correlated with the reduction in the levels of inflammatory cytokines exerted by these nanomaterials [39] and/or their antioxidant properties. Accordingly, the current study suggested the protective role of CAE-SNs, Hst-SNs and Nrg-SNsnanomaterials against AA toxicity in terms of brain damage as well as cytokine levels [39].

Polyphenolic compounds have diverse in chemical structure and characteristics [5]. The role of flavonoids, including Hsd and Hst in human brain health has been reported as they enhance learning and memory. They

may have a role in elevating brain-derived neurotrophic factor and reversing the disruptive effect of global cerebral I/R on memory [3, 6].

Dietary compounds like flavonoids may offer protection against neurodegeneration. Flavonols such as quercetin and catechin showed potential protective effects on cell integrity and morphological lesions in *in vitro* primary neuronal cultures, under copper-induced oxidative stress conditions [5]. Co-administration of other naturally occurring flavonolaglycone (quercetin) reverses the behavioral and biochemical changes in neuroleptic-induced orofacial dyskinesia [40]. In the present work, the protective effect of the investigated nanoparticles of flavanones (Hst-SNs and Nrg-SNs) may have resulted from its ability to enhance the antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and reduce inducible nitric oxide synthase (iNOS) activity [41]. The *in vivo* results of the current study indicated that flavonoids, including flavones and flavanones are the promising natural products for protecting neuronal cells from neurotoxicity and oxidative stress, and the administration of the flavanones-nanoparticles (Hst-SNs and Nrg-SNs) could be a feasible therapeutic strategy for protecting the aging brain or slowing neurodegenerative processes.The treatment of flavones luteolin has been showing marked role in improving the stress-induced cognitive impairments, decreased GSH concentration and SOD activities in prefrontal cortex and hippocampus [42]. Oxidative stress plays an important role in the pathogenesis of early brain injury. The antioxidant flavonol-3-*O*-glycosides were reported to inhibit the production of ROS, NO, GSH disulfide, MDA [41]. A study suggested the involvement of free radicals in the development of neuroleptic-induced orofacial dyskinesia and role of the flavone glycosides to treat this hyperkinetic movement disorder [43]. In addition the present results clearly demonstrated that Hst-SNs nanomaterial. The flavanone Hst has been reported as a neuroprotective agent against the neurotoxicity exerted by H_2O_2 and on amyloid β, 3-nitropropionic acid [44].

In addition, AA administration resulted in significant reductions in the enzymatic antioxidant parameters of the brain; SOD and CAT. These results suggest that Nrg-SNs and Hst-SNs may have beneficial role against AA-induced oxidative stress in the brain; an effect that is mainly attributed to the antioxidant property of Nrg and Hst.

In the present work, the reported neuroprotective effects of flavanone-basenanomaterials (Hst-SNs and Nrg-SNs) may be attributed to improvement of neuronal energy metabolism and reversing mitochondrial dysfunction [45] or the beneficial effects on nervous system by the inhibition of nitrosative stress and nitrergic pathway.

Many studies have proved that the plant extracts act as a potential precursor for the synthesis of nanomaterial in non-hazardous ways and with the advantages of high stability, high carrying capacity, and variable routes of administration [46]. It summary, AA induced brain damage which might be related to oxidative stress. Administration of the three selected nano-components lessened the negative effects of AA on the brain by inhibiting free radical mediated process; an effect that could be attributed to the antioxidant properties of the three selected nano-components.Hence, this process could be suitable for developing a biological process for mass scale production of nanoparticles.

These results provide evidence for the neuroprotective effect of both fruit waste and flavanones nanomaterials. They were able to significantly alleviate the neurotoxicityinduced by AA in Wistarratsby reducing oxidative stress. Oral administration of Hst was reported as a shown protecting agent against AA-induced brain damage and oxidative stress [47]. Naringenin Nrg, one of the abundant citrus flavonoid, was proved to possess antioxidative activity in high cholesterol-fed rabbits through improving the plasma lipid levels and increasing the plasma antioxidant activity [11]. Moreover, Nrg can protect the rat liver and brain against the radiation-induced damage by elevating the antioxidant status and reducing the lipid peroxidation [14]. In the current study Hst-SNs was suggested to promote cellular antioxidant defence systems by its properties as a free radical scavenger [9]. It may prevent the oxidative stress and other adverse effects caused by the toxic agent acrylamide that lead to oxidative damage. Nrg and Hst showed potential for use in neurodegenerative intervention. From the present results, it can be concluded that the neuroprotective effects of fruit waste material and citrus flavanone aglycones Hst and Nrg against AA-induced toxicationand oxidative stress may be due to the inhibition of oxidative stress overproduction and maintenance of antioxidant defence mechanisms.

3.4. Histopathological analysis

3.4.1. Effect of CAE-SNs, Hst-SNs and Nrg-SNs as well as donepezil HCl on brain morphology

In the control group of rats, normal histopathological features in the brain tissue (cerebellum) were observed and molecules granular were well defined. Purkinje layers were also marked with closely packed cells (Figure 1).

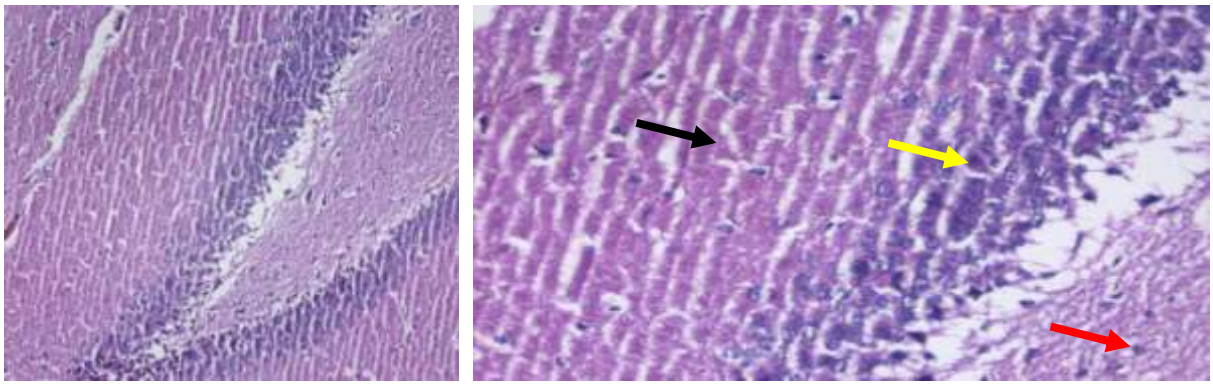

Figure 1: Photomicrograph of hematoxylin and eosin stained section of brain of normal control rats, showed the cerebellum with normal histological features, illustrating a well defined molecular (black arrow), granular (red arrow) and Purkinje layers (yellow arrow) and presence of numerous closely packed small cells in the granular layer as well a large Purkinje cells in the Purkinje cell layer. The normal structure of neuronal cells (H and E stain, x200, x400) was shown.

Cellular degeneration of the cerebellum was recorded in the brain section of AA-intoxicated rats and atrophy. The cell number was decreased in Purkinje and granular layers. Neuronal atrophy with perineuronalvacuolation and oedema were shown (Figure 2).

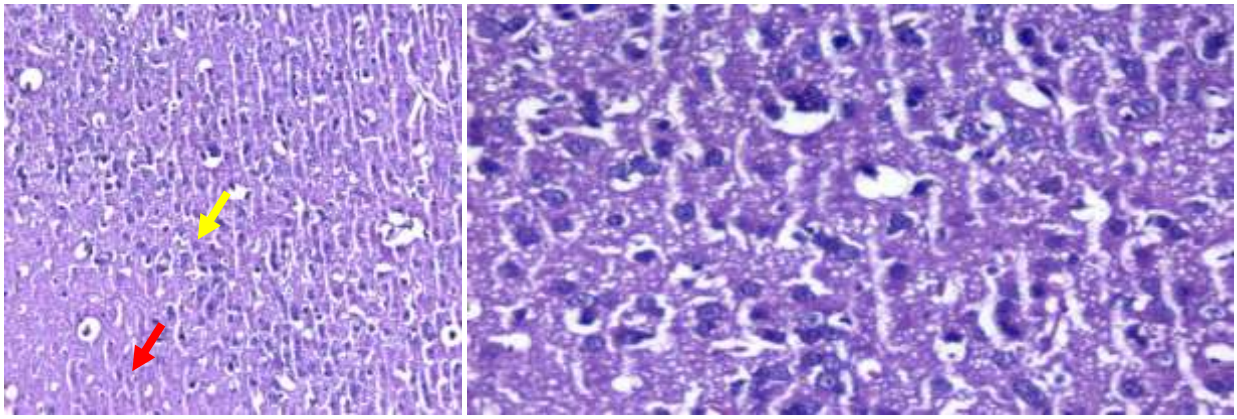

Figure 2: Photomicrograph of hematoxylin and eosin stained section of brain of AA intoxicated rats, showed the cerebellum with cellular degeneration and atrophy thus leading to a decrease in the number of cells in the granular and Purkinje layers respectively (black and red arrows) or showing oedema and interstitial neuronal atrophy with perineuronalvacuolation (H&E,x200,x400)

Normal histological features in the brain section (cerebellum) of the normal, healthy group treated with nanomaterial of CAE-SNs, Hst-SNs and Nrg-SNs were marked. The granular, molecular, Purkinje layers were defined well. In the granular layer, large numbers of small cells were packed closely. In the Purkinje cell layer, there was numerous Purkinje cells were present or normal structure of neuronal cells (Figures 3,4,5).

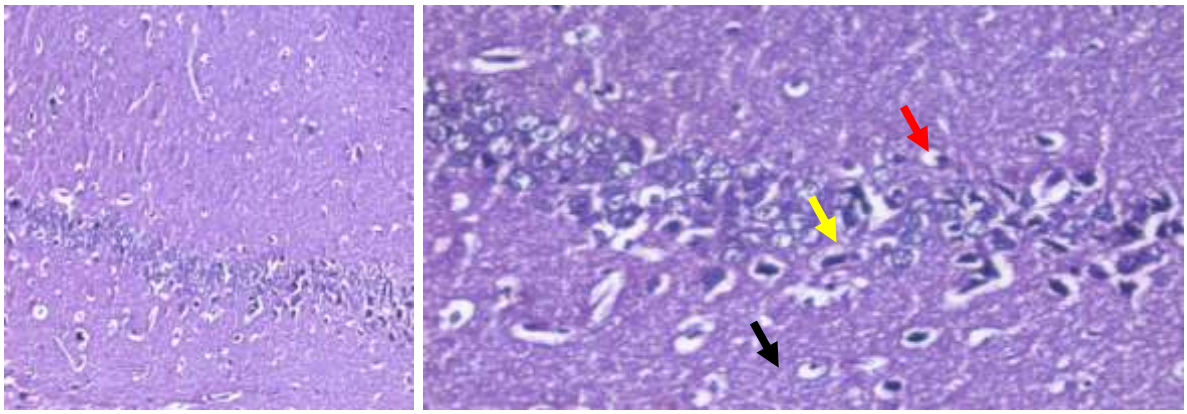

Figure 3: Photomicrograph of hematoxylin and eosin stained section of the brain of rats treated with citrus nanoparticle, showed the cerebellum with normal histological features, illustrating a well-defined molecular (black arrow), granular (red arrow) and Purkinje layers (yellow arrow) and presence of numerous closely packed small cells in the granular layer as well a large Purkinje cells in the Purkinje cell layer.

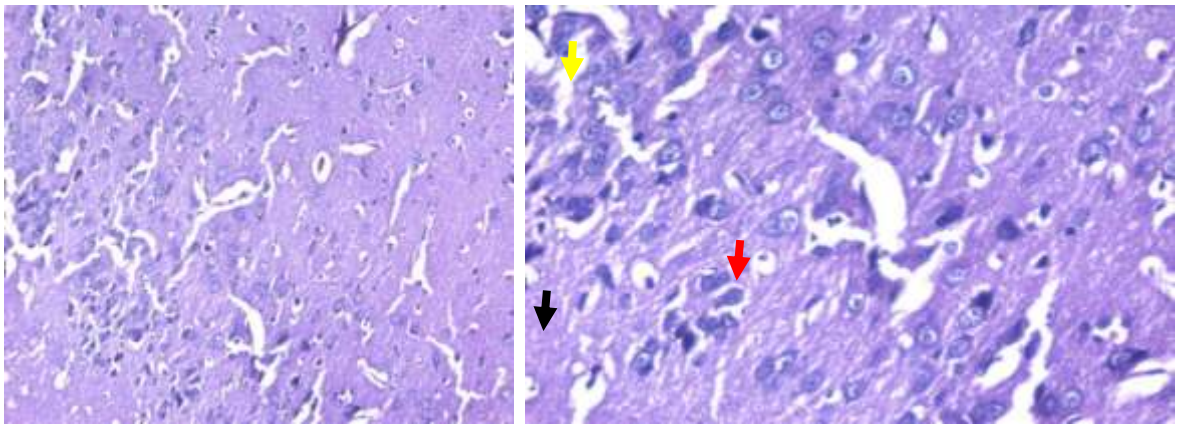

Figure 4: Photomicrograph of hematoxylin and eosin stained section of the brain of rats treated with Hst-SNs, showed the cerebellum with normal histological features, illustrating a well defined molecular (black arrow), granular (red arrow) and Purkinje layers (yellow arrow) and presence of numerous closely packed small cells in the granular layer as well a large Purkinje cells in the Purkinje cell layer and/or normal structure of neuronal cells (H&E stain, x 200, x400).

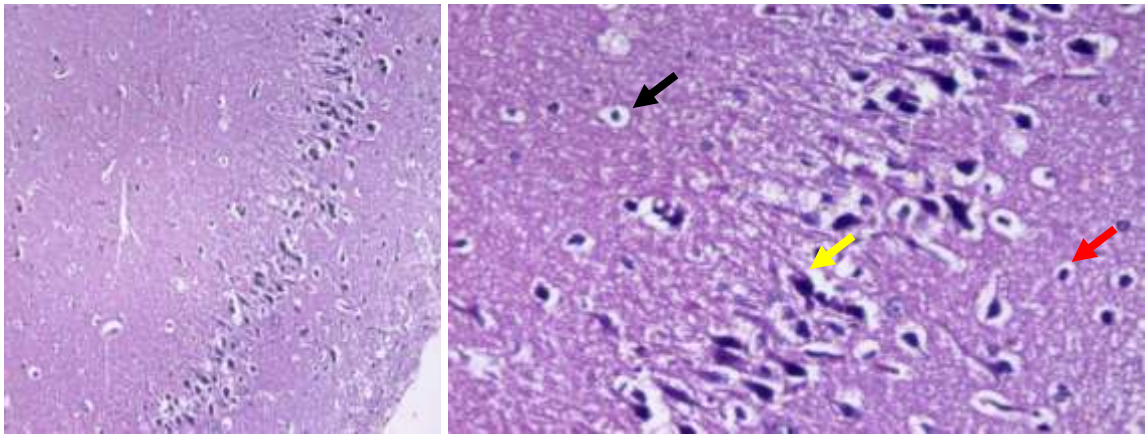

Figure 5: Photomicrograph of hematoxylin and eosin stained section of the brain of rats treated with Nrg-SNs, showed the cerebellum with normal histological features, illustrating a well-defined molecular (black arrow), granular (red arrow) and Purkinje layers (yellow arrow) and presence of numerous closely packed small cells in the granular layer as well a large Purkinje cells in the Purkinje cell layer and/or normal structure of neuronal cells (H&E stain, x 200, x400).

Histological features of the cerebellum were normal in the brain section of rats treated with Hst-SNs and Nrg-SNs administered post-AA. Mild decrease granular and well defined molecular and Purkinje layers were observed. In the granular layer, large number of small cells were packed closely were also detected. In the Purkinje cell layer, there was numerous Purkinje cells were present or normal structure of neuronal cells (Figures 6,7).

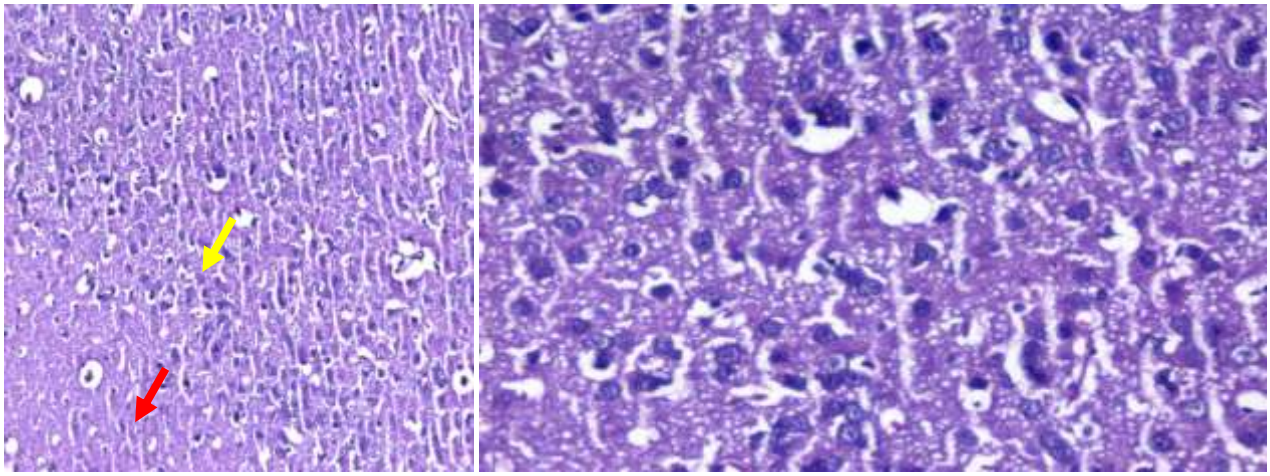

Figure 6: Photomicrograph of hematoxylin and eosin stained section of the brain of rats treated with CAE-SNs post AA administration showed the cerebellum with cellular degeneration and atrophy thus leading to a decrease in the number of cells in the granular and Purkinje layers respectively (H and E, x200 and x400).

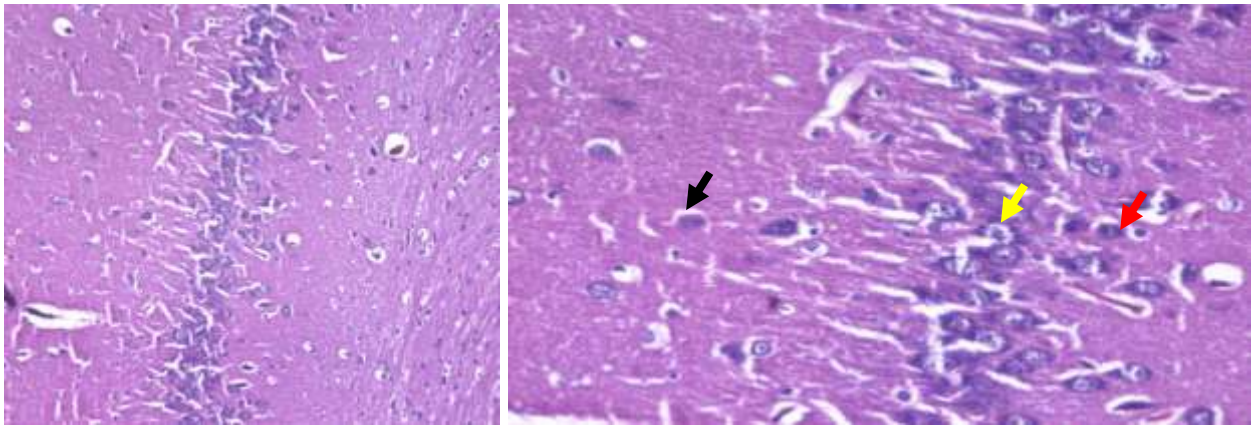

Fig. 7: Photomicrograph of hematoxylin and eosin stained section of the brain of rats treated with Hst-SNs post AA administration, showed the cerebellum with normal histological features, illustrating a well defined molecular (black arrow) mild decrease in granular (red arrow) and Purkinje layers (yellow arrow) and presence of numerous closely packed small cells in the granular layer as well a large Purkinje cells in the Purkinje cell layer or showing the normal structure of neuronal cells (H and E stain, x200 and x400).

The cerebellum of the rats treated with CAE-SNs nanomaterial showed atrophy and cellular degeneration. In the Purkinje and granular layers, the cells were in low account number (Figure 8).

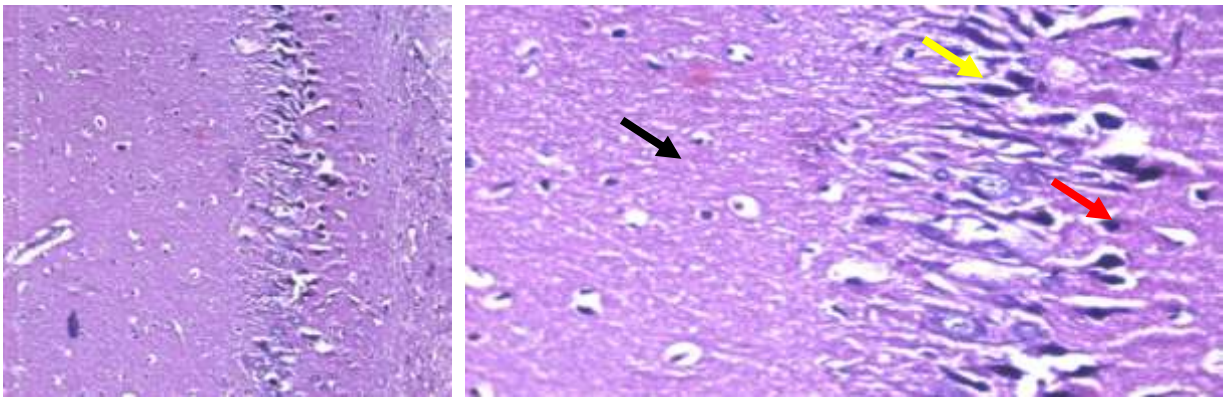

Figure 8: Photomicrograph of hematoxylin and eosin stained section of the brain of rats treated with Nrg-SNs post AA administration, showed the cerebellum with normal histological features, illustrating a well-defined molecular (black arrow), mild decrease in granular (red arrow) and Purkinje layers (yellow arrow) and presence of numerous closely packed small cells in the granular layer as well a large Purkinje cells in the Purkinje cell layer. The normal structure of neuronal cells (H and E stain, x200, x400) was shown.

The cerebellum was of normal histological features as indicated in the brain section of donepezil HCl-administered post AA treatment. A well-defined molecular, granular and Purkinje layers and presence of numerous closely packed small cells in the granular layer were present. In the Purkinje cell layer, there was large Purkinje cells were present or normal structure of neuronal cells (Figure 9).

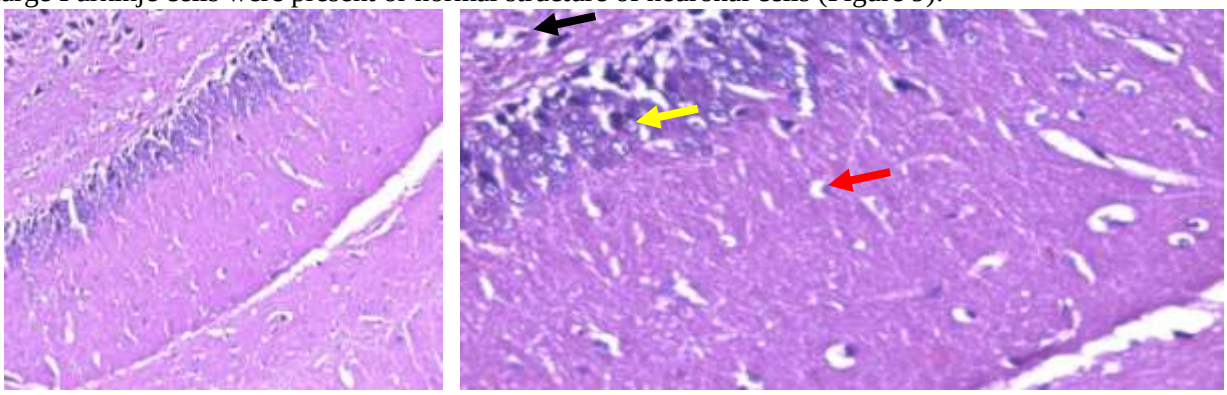

Figure 9: Photomicrograph of hematoxylin and eosin stained section of the brain of rats treated with donepezil HCl post AA administration, showed the cerebellum with normal histological features, illustrating a well-defined molecular (black arrow), granular (red arrow) and Purkinje layers (yellow arrow) and presence of numerous closely packed small cells in the granular layer as well a large Purkinje cells in the Purkinje cell layer, with mild interstitial neuronal atrophy or the normal structure of neuronal cells (H and E stain, x200, x400) was shown.

In summary, although the treated with nanoparticles of CAE-SNs, Hst-SNs and Nrg-SNs showed nearly the same improvement in brain architecture, but the nanoparticles of Hst-SNs and Nrg-SNs recorded the most marked enhanced picture. Treatment with flavanones restored tissue and serological indices concomitantly towards normal levels. The treatment with Hst-SNs and Nrg-SNs ameliorated the functional and histological outcomes with elevated endogenous antioxidants status.

Conclusion

CAE-SNs, Hst-SNs and Nrg-SNs administrated to acrylamide intoxicated rats have the abilities to down regulate free radicals elevation, improve brain functions, ameliorate brain neurotransmitters as well as the normalize brain cell architectures. From the present results, it can be concluded that the neuroprotective effects of fruit waste material and citrus flavanoneaglyconesHst and Nrg against AA-induced toxication and oxidative stress may be due to the inhibition of oxidative stress overproduction and maintenance of antioxidant defence mechanisms. So, these fruit waste and flavanones nanomaterials may be used as a new safe therapy that may delay the brain injuries progression and their complications.

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