*Available online at* www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

**Der Pharma Chemica, 2017, 9(2):99-108**
*(http://www.derpharmachemica.com/archive.html)*

# Healing Potency of Citrus, Hesperetin and Naringenin Loaded with Silicate Nanoparticles on Neurotoxicity Induced by Acrylamide Toxic Dose

**Maha Z. Rizk[1], Abo-elmatty D.M[2], Hanan F. Aly[1]*, Howaida I. Abd-Alla[3], Samy M. Saleh[2], Eman A.Younis[1]**

*[1]Department of Therapeutic Chemistry, National Research Centre, Dokki 12622, Giza, Egypt*
*[2]Department of Biochemistry, Faculty of Pharmacy, Suez Canal University, Egypt*
*[3]Chemistry of Natural Compounds Department, National Research Centre, Giza, Dokki 12622, Egypt.*

**ABSTRACT**

*The current research demonstrated the effect of silica loaded nanoparticles (citrus, naringenin and hesperetin) on acrylamide (ACR) intoxicated rats, through measuring specific selected biomarkers on brain. Brain neurotransmitters noradrenaline (NA), adrenaline (A), dopamine (DA) and serotonine (5-HT), acetylcholine esterase (ACHE) andcaspase-3 enzyme activity and DNA degradation (Comet) were evaluated. ATPase enzyme activity and total protein content were also evaluated. Besides, the presented data showed significant deterioration in the all selected biomarkers investigation in aclylamide-intoxicated rats. It showed significant reduction in brain neurotransmitters (NAADA and 5-HT), ATPase enzyme activity and total protein content while significant elevation in AchE and Caspase-3 enzyme activity and DNA damage. Treatment of intoxicated rats with the three silica loaded nanoparticles indicated amelioration with different percentage of improvement in the all measured biomarkers as well as in rat's brain architecture. Thus it could be concluded that, the using of natural product nanoparticles can up-regulate brain neurotransmitters, suppressing DNA damage, diminished brain damage that leads to delay disease development and/or its complication.*

**Keywords**: Acrylamide, ATPase, Brain neurotransmitters, Comet assay

## INTRODUCTION

Acrylamide is a reactive monomer used in many technological applications, but it is the incidental formation during cooking of common starchy foods that leads to pervasive human exposure, typically in the range of 1 µg/Kg body weight (bw)/day (d) [1].

Acrylamide formation was found to occur during the browning process by the Maillard reaction of reducing sugars with the aminoacid asparagine at temperatures higher than 120ºC [2]. Acrylamide, a human neurotoxicant and rat tumorigenic [3]. Evidence has been reported that acrylamide is neurotoxic [4,5] among over-exposed humans and is amulti-site carcinogen in rats exposed by ingestion over a lifetime [6,7].

Exposure to neurotoxic agents is a common event in the workplace and in the general environment. The already large number of neurotoxic substances [8] is constantly increasing with newly generated compounds as they needed for a rapidly changing and growing market [9]. Risk assessment is a fundamental process for prevention, especially for neurotoxicity [10]. Acrylamide neurotoxicity in both laboratory animals and humans is characterized by ataxia and distal skeletal muscle weakness. In addition, acrylamide intoxication in rodent models is associated with selective nerve terminal damage in both central and peripheral nervous systems [11]. A growing body of evidence indicated that the nerve terminal is a primary site of acrylamide action and that inhibition of corresponding membrane fusion processes impaired neurotransmitter release and promoted eventual degeneration [12]. This might be attributed to, easily absorption by all routes of administration, and both the Central Nervous System (CNS) and Peripheral Nervous System (PNS) are the selective targets for its toxicity. Furthermore, acrylamide induced neurotoxic effects discernible in terms of diminished ATPase activity, enhanced activity of acetylcholine esterase and dopamine, noradrenaline, adrenaline, serotonine depletion. Marked enhanced DNA damage and caspase-3- activity [13,14]. During the past decade, there has been a rapid progress on research in the areas of nano-science and nanotechnology. Nanomaterial has specific properties, such as small size, large surface area, shape, and special structure [15]. Because of the unique dimensional and morphological properties, nanomaterial can be physically and chemically manipulated and widely used in industrial and biomedical processes as well as organ or cell specific

drug delivery [16]. The rapid growth of nanotechnology industry has led to the wide-scale production and application of engineered NPs. Moreover, nano-sized crystalline $SiO_2$ is commonly used in semiconductor manufacture [17].

Natural dietary components include vegetables, fruits and spices; they have drawn an immense attention due to their ability to suppress various phases of carcinogenesis and neurodegenerative intervention because of the presence of phytochemicals [18]

Natural flavonoids with more of a lipophilic chemical structure and antioxidant properties are promising candidates for neurodegenerative intervention [19]. On the other hand, *Citrus aurantium*, a fruit commonly known as bitter orange, it belongs to the Rutaceae family. Citrus fruits are an abundant source of various flavonoids, which have been used as a traditional herbal medicine in Korea and China [20]. Naringenin is an important natural flavanone. It is known to be a multi-functional agent, a powerful anti-oxidant, anti-inflammatory, antitumor, anti-depressant, hepatoprotective and neuroprotective compound [21]. Also naringinen was known to exhibit free-radical scavenging activity [22]. Moreover, Hesperetin (Hsp) flavanone is the major circulating aglycone metabolite of hesperidin, abundant in citrus fruit and drinks [23]. Hsp has been shown to be a potential anti-oxidant, anti-inflammatory, neuroprotective agent [24]. Hence, the present study is designed to examine total ethanol extract of Citrus aurantium beside two citrus flavonoids as naringenin and hesperetin isolated from Citrus aurantium (sour orange) family Rutaceae to ameliorate neurotoxicity induced by acrylamide in experimental rat's model.

## MATERIALS AND METHODS

### Fruit materials

The fruits were obtained from Banha, Qalyubia governorate, Egypt. All the fruits were of eating quality, and without blemishes, or damage. The pericarp region (peel) is separated from the edible part. The white, spongy inner part of the peel, called the mesocarp, or albedo was separated, dried at room temperature and grinded.

*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), preparation of the nanomaterials based on Tetraethoxyorthosilicate (TEOS).

*Isolation of flavanones (naringinen and hesperetin)*

Naringenin [25] and hesperetin [26] were done on the basis of chromatographic properties, 1H and 13C NMR spectroscopic data with literature values.

*Preparation of the nanomaterial based on Tetraethoxyorthosilicate (TEOS)*

It is prepared according to Haron et al., [27] unpublished data.

*Animals*

Male Wistar albino rats (100: 120 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet.

*Ethics*

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

*Doses and route of administration*

CR suspended in $H_2O$ and was injected intraperitoneal in a dose of 50 mg/kg body weight five times weekly for ten consecutive days [11]. SOAE, hesperetin and naringenin nanoparticles were administered orally five times weekly for one month and half post ACR induction in a dose 100 mg/kg body weight [28,29]. Donepezil-HCl, a reference drug, was administered orally five times weekly for one month and half post ACR treatment in a dose 50 mg/kg body weight [30].

## 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. Group 1: Will be normal healthy control rats. Groups 2-4: Will be normal healthy rats orally administrated with silica loaded nanoparticles: Citrus (SOAE), naringenin and hesperetin for one month and half, respectively. Group 5: Will be injected intrapretonial with toxic dose of ACR for 10 days and served as intoxicated group. Groups 6 and 8: Will be orally administrated with silica loaded nanoparticles: SOAE, naringenin and hesperetin post ACR administration respectively for one month and half. Group 9: Will be orally administrated with donepezil-HCl as reference drug post ACR administration for one month and half.

### Determination of brain biomarkers

*Estimation of brain neurotransmitters*

Determination of adrenaline: Adrenaline activity was measured by a quantitative enzyme-linked immune sorbent assay (ELISA)

technique according to the manufacturer's instructions [31].

Determination of noradrenaline, dopamine and serotonin: Brain NA, DA and 5-HT were determined using high performance liquid chromatography HPLC) technique according to Zagrodzka [32].)

*Estimation of acetylcholine esterase activity*

Serum acetyl cholinesterase was measured by a quantitative enzyme-linked immunosorbent assay (ELISA) technique according to Wen [33].

*Estimation of brain caspase-3 activity*

Brain caspase-3 activity was measured by a quantitative enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions Savitha [34].

*Estimation of DNA damage percent by Comet assay in brain tissues*

Single cell gel electrophoresis assay (also known as comet assay) was performed as previously described by Singh [35].

*Estimation of ATPase activity in brain*

ATPase in brain was estimated according to the method of Matteucci [36].

*Estimation of brain total protein content*

Total protein content was estimated according to the method of Bradford [37].

## RESULTS AND DISCUSSION

*Effect of SOAE, hesperetin and naringenin loaded with silicatenanoparticles on noradrenaline and adrenaline neuro-transmitters*

SOAE, hesperetin and naringenin nanoparticles administered to healthy control rats showed insignificant change in noradrenaline and adrenaline levels as compared to control untreated rats. While, acrylamide intoxicated rats showed significant reduction in both noradrenaline and adrenaline levels with reduction percentages 47.81 and 59.00%, respectively as compared to control rats. Treatment of intoxicated rats with SOAE, hesperetin, naringenin nanoparticles as well as donepezil reference drug showed significant increase in noradrenaline and adrenaline levels comparing to intoxicated rats. However, significant decrease was detected in noradrenaline level comparing to normal control rats with improvement percentages 29.20%, 28.00%, 30.62%, and 33.71%, respectively. While, insignificant difference in adernaline level was detected post treatment of acrylamide-induced rats with the different remediation types comparing to healthy control rats (Table 1).

**Table 1: Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on brain noradrenaline and adrenaline levels in acrylamide -induced rats**

| Groups / Parameters | Control (-ve) | Treated controls | | | Acrylamide (+ve) | Acrylamide- treated | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SOAE | Hesperetin | Naringenin | | SOAE | Hesperetin | Naringenin | donepezil -HCl |
| NA Mean | 187.86 ± 7.47$^{b}$ | 182.89 ± 6.20$^{b}$ | 185.63 ± 6.45$^{b}$ | 189.26 ± 4.99$^{b}$ | 98.08 ± 6.17$^{l}$ | 152.97 ± 5.12$^{g}$ | 150.67 ± 5.56$^{g}$ | 155.59 ± 7.59$^{g}$ | 161.41 ± 6.66$^{g}$ |
| % Change compared to control rats | | 2.61 | 1.20 | 0.70 | 47.81 | 18.60 | 19.80 | 17.21 | 14.10 |
| % Change compared to intoxicated rats | | | | | | 55.96 | 53.62 | 58.64 | 64.55 |
| % of Improvement | | | | | | 29.20 | 28.00 | 30.62 | 33.71 |
| A Mean | 292.12 ± 12.21$^{e}$ | 284.31 ± 11.06$^{e}$ | 283.09 ± 12.26$^{e}$ | 292.76 ± 12.39$^{e}$ | 119.75 ± 9.23$^{g}$ | 303.25 ± 12.61$^{e}$ | 312.42 ± 14.15$^{e}$ | 319.42 ± 11.17$^{e}$ | 298.93 ± 12.64$^{e}$ |
| % Change compared to control rats | | 2.70 | 3.12 | 0.21 | 59.00 | 3.81 | 6.91 | 9.31 | 2.32 |
| % Change compared to intoxicated rats | | | | | | 153.24 | 160.89 | 166.74 | 149.63 |
| % of Improvement | | | | | | 62.81 | 66.00 | 68.40 | 61.30 |

Data are means ± SD of six rats in each group. Data are expressed as ng/g for NA and A. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version, 8), where unshared letters between groups are the significance value at $p \leq 0.05$

Regarding to the recorded changes in noradrenaline, donepezil-HCl reference drug administered post acrylamide showed the highest percentage of improvement by 33.71%, followed by naringenin nanopraticle administered post acrylamide which recorded improvement percentage 30.62% and SOAE nanoparticle (29.20%). While, hesperetin nanoparticle administered post acrylamide intoxicated rats recorded the lowest improvement value (28.00%) (Table 1).

*Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on serotonine and dopamine-HCl neurotransmitterlevels*

In addition SOAE, hesperetin and naringenin nanoparticle administered to healthy rats showed insignificant difference in brain serotonine and dopamine levels as compared to untreated control rats. However, acrylamide intoxicated rats showed significant decrease in serotonine and dopamine levels by 41.71% and 24.31%, respectively. Treatment of acrylamide induced rats with SOAE, hesperetin, naringenin nanoparticles as well as donepezil-Hcldeclared significant increase in serotonine and dopamine levels as compared to intoxicated rats. While, significant decrease in brain serotonine level was detected upon treated intoxicated rats with SOAE, hesperetin, naringenin nanoparticles as well as donepezil comparing to control rats with amelioration percentages 19.40%, 21.71%, 24.40%, and 27.62%, respectively. For dopamine, significant increase was detected comparing to control rats with amelioration percentages 48.70%, 39.52%, 45.41%, and 35.42%, respectively for SOAE, hesperetin, naringenin nanoparticles as well as donepez Regarding to the recorded changes in serotonine, donepezil administered post acrylamide showed the highest percentages of improvement (27.62%), followed by naringenin (24.40%). While for dopamine, SOAE nanoparticle recorded the highest improvement percentage (48.70%), followed by naringenin (45.41%) (Table 2).

**Table 2: Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on serotonine and dopamine-HCl neurotransmitter levels**

| Groups / Parameters | Control (-ve) | Treated controls | | | Acrylamide (+ve) | Acrylamide- treated | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SOAE | Hesperetin | Naringenin | | SOAE | Hesperetin | Naringenin | donepezil HCl |
| 5-HT Mean | 86.01 ± 3.08$^{a}$ | 83.25 ± 2.16$^{a}$ | 86.16 ± 2.03$^{a}$ | 85.29 ± 2.12$^{a}$ | 50.12 ± 0.38$^{g}$ | 66.81 ± 2.34$^{e}$ | 68.80 ± 2.44$^{e}$ | 71.09 ± 2.41$^{d}$ | 73.85 ± 2.06$^{d}$ |
| % Change compared to control rats | | 3.20 | 0.17 | 0.80 | 41.71 | 22.32 | 20.00 | 17.31 | 14.10 |
| % Change compared to intoxicated rats | | | | | | 33.30 | 37.27 | 41.84 | 47.35 |
| % of Improvement | | | | | | 19.40 | 21.71 | 24.40 | 27.62 |
| DA Mean | 59.43 ± 3.21$^{ef}$ | 60.79 ± 3.33$^{e}$ | 58.23 ± 2.17$^{f}$ | 55.37 ± 2.23$^{ef}$ | 44.99 ± 2.73$^{H}$ | 73.95 ± 2.08$^{a}$ | 68.45 ± 2.83$^{d}$ | 71.99 ± 2.09$^{a}$ | 66.04 ± 2.66$^{d}$ |
| % Change compared to control rats | | 2.30 | 2.00 | 6.82 | 24.31 | 24.41 | 15.20 | 21.10 | 11.11 |
| % Change compared to intoxicated rats | | | | | | 64.37 | 52.14 | 60.01 | 46.79 |
| % of Improvement | | | | | | 48.70 | 39.52 | 45.41 | 35.42 |

Data are means ± SD of six rats in each group. Data are expressed as ng/g. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version 8), where unshared letters between groups are the significance value at $p \leq 0.05$

*Effect of SOAE, hesperetin and naringenin loaded with silicatenanoparticles on acetylcholine esterase enzyme activity (AchE).*

Moreover, control rats treated with SOAE, hesperetin, naringenin nanoparticles demonstrated insignificant change in AchE activity as compared to control untreated rats. Acrylamide-induced rats declared significant increase in AchE activity (24.30%), comparing to control rats. On the other hand, intoxicated rats treated with SOAE, hesperetin, naringenin nanoparticles as well as standard drug showed significant reduction in AchE activity either comparing to control rats or acrylamide induced rats with percentages of improvement 45.51%, 50.90%, 47.60%, and 35.90%, respectively for SOAE, hesperetin, naringenin nanoparticles as well as standard drug. Hence, hesperetin recorded the highest improvement percentage (50.90%), followed by naringenin nanoparticle (47.60%) (Table 3).

*Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on caspase-3 enzyme activity.*

Further, Table 4 demonstrated insignificant difference in caspase-3 activity between control rats treated with nanoparticles and untreated control one. While, as compared to normal healthy rats, acrylamide intoxicated rats showed significant increase in caspase-3 enzyme activity (119.80%). On the other hand, acrylamide–induced rats treated with SOAE, hesperetin, naringenin nanoparticles and donepezil–HCl drug recorded significant decreased in enzyme activity as compared intoxicated rats. However, significant increase in enzyme activity was detected upon treated intoxicated rats with nanoparticles as well as standard drug comparing to control rats with amelioration percentages 76.91, 68.36, 82.91 and 78.71%, respectively for SOAE, hesperetin, naringenin and donepezil-HCl drug. So, naringenin nanoparticle administered post acrylamide showed the highest improvement percentage in caspase-3 activity (82.91%), followed by donepezil HCl (78.71%) (Table 4).

**Table 3: Effect of citrus, hesperetin and naringenin loaded with silicate nanoparticles on acetylcholine esterase enzyme (AchE) activity in acrylamide –induced rats**

| Groups / Parameters | Control (-ve) | Treated controls | | | Acrylamide (+ve) | Acrylamide treated | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SOAE | Hesperetin | Naringenin | | SOAE | Hesperetin | Naringenin | donepezil HCl |
| AchE Mean | 82.01 ± 2.08$^{b}$ | 79.90 ± 1.16$^{b}$ | 81.45 ± 2.47$^{b}$ | 85.24 ± 2.34$^{b}$ | 101.94 ± 1.27$^{h}$ | 64.61 ± 3.62$^{e}$ | 60.21 ± 3.68$^{e}$ | 62.93 ± 3.43$^{e}$ | 72.52 ± 3.69$^{b}$ |
| % Change compared to control rats | | 2.60 | 0.71 | 3.92 | 24.30 | 21.20 | 26.62 | 23.31 | 11.61 |
| % Change compared to intoxicated rats | | | | | | 36.62 | 40.94 | 38.27 | 28.86 |
| % of Improvement | | | | | | 45.51 | 50.90 | 47.60 | 35.90 |

Data are means ± SD of six rats in each group. Data are expressed as ng/ml for AchE. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version 8), where unshared letters between groups are the significance value at $p \leq 0.05$

**Table 4: Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on caspase-3 enzyme activity in acrylamide induced rats**

| Groups / Parameters | Control (-ve) | Treated controls | | | Acrylamide (+ve) | Acrylamide treated | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SOAE | Hesperetin | Naringenin | | SOAE | Hesperetin | Naringenin | donepezil HCl |
| Caspase -3 Mean | 6.02 ± 0.52$^{g}$ | 5.66 ± 0.44$^{g}$ | 6.33 ± 0.53$^{g}$ | 5.95 ± 0.66$^{g}$ | 13.23 ± 0.95$^{a}$ | 8.60 ± 0.34$^{ce}$ | 9.12 ± 0.22$^{c}$ | 8.24 ± 0.44$^{e}$ | 8.49 ± 0.42$^{ce}$ |
| % Change compared to control rats | | 6.00 | 5.10 | 1.22 | 119.80 | 42.92 | 51.51 | 36.90 | 41.00 |
| % Change compared to intoxicated rats | | | | | | 35.00 | 31.07 | 37.72 | 35.83 |
| % of Improvement | | | | | | 76.91 | 68.30 | 82.91 | 78.71 |

Data are means ± SD of six rats in each group. Data are expressed as ng/100 mg. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version 8), where unshared letters between groups are the significance value at $p \leq 0.05$

*Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on on DNA tail length (Comet assay) activity*

Table 5 markedly indicated significant reduction in DNA tail length represented by Comet assay (15.91 and 13.10% respectively), upon treated control rats with SOAE and hesperetin nanoparticles while, naringenin nanoparticle showed an insignificant decrease compared to untreated control one. Treatment of intoxicated rats with SOAE, hesperetin and naringenin nanoparticles as well as donepezil-HCl standard drug demonstrated, significant reduction in DNA tail length comparing to acrylamide induced rats, while significant increase in DNA tail length was recorded comparing to healthy control rats with improvement percentages 73.41, 71.80, 59.12 and 77.41%, respectively, for SOAE, hesperetin, naringenin nanoparticles as well as donepezil-HCl drug. Hence, donepezil-HCl showed the highest improvement percentage (77.41%), followed by SOAE nanoparticle (73.41%), (Table 5).

The % of the tailed DNA in control group was 5.00% of the total DNA counts. Normal healthy rats treated with nanoparticles of SOAE, hesperetin and naringenin showed tailed DNA percentages represented by 6.00, 4.00 and 5.00% respectively which are statistically insignificant comparing to control rats. Acrylamide intoxicated group showed significant increase in tailed DNA (18.00%). Rats treated with nanoparticles of SOAE, hesperetin, and naringenin and donepezil-HCl drug post acrylamide treatment showed significant increase in tailed DNA percentages represented by 14.00%, 15.00%, 12.00%, and 11.00%, respectively as compared to control. In case of un-tailed DNA, it represented by 95.00% in control group. Normal healthy rats treated with nanoparticles of SOAE, hesperetin and naringenin showed insignificant un tailed DNA percentages 94.00, 96.00 and 95.00%, respectively. While, in acrylamide intoxicated group it represented by 82.00%. Rats treated with nanoparticles of SOAE, hesperetin, naringenin and donepezil-HCl post acrylamide treatment showed significant increase in un tailed DNA comparing to acrylamide–induced rats, however significant decrease comparing to control one represented by 86.00, 85.00, 88.00 and 89.00%, respectively of un tailed DNA. The total DNA percentage was represented by 2.08% for control. While, the total percentages of DNA in normal healthy rats treated with nanoparticles of SOAE, hesperetin and naringenin reached to 2.03%, 2.46%, and 2.28%, respectively. However, in acrylamide intoxicated group, the total DNA was represented by 5.02%. On the other hand, the total DNA in rats treated with nanoparticles of SOAE, hesperetin, naringenin and donepezil-HCl standard drug post acrylamide treatment recoded significant decrease comparing to intoxicated rats, while significant increase comparing to control rats reached to 3.66%, 3.36%, 3.09%, and 3.22%, respectively. The tail moment, which is represented by tail length × total DNA, was expressed by 5.30 units in control rats.

**Table 5: Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on DNA tail length (Comet assay) activity in acrylamide induced rats**

| Groups / Parameters | Control (-ve) | Treated controls | | | Acrylamide (+ve) | Acrylamide treated | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SOAE | Hesperetin | Naringenin | | SOAE | Hesperetin | Naringenin | donepezil Hcl |
| Comet assay Mean | 2.52 ± 0.02$^{e}$ | 2.12 ± 1.01$^{g}$ | 2.19 ± 0.01$^{f}$ | 2.49 ± 0.01$^{e}$ | 4.98 ± 0.07$^{a}$ | 3.13 ± 0.01$^{c}$ | 3.17 ± 0.01$^{c}$ | 3.49 ± 0.01$^{b}$ | 3.03 ± 0.02$^{d}$ |
| % Change compared to control rats | | 15.91 | 13.10 | 1.22 | 97.60 | 24.23 | 25.81 | 38.50 | 20.20 |
| % Change compared to intoxicated rats | | | | | | 37.15 | 36.35 | 29.92 | 39.16 |
| % of Improvement | | | | | | 73.41 | 71.80 | 59.12 | 77.41 |

Data are means ± SD of six rats in each group. Data are expressed as µm. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version 8), where unshared letters between groups are the significance value at $p \leq 0.0$

While in normal healthy rats treated with nanoparticles of SOAE, hesperetin and naringenin recorded tail moment by 4.43, 5.39 and 5.72 units, respectively. In acrylamide intoxicated rats, it was represented by 24.50%. Rats treated with nanoparticles of SOAE, hesperetin, naringenin and donepezil HCl post acrylamide treatment recorded significant reduction in tail moment comparing to acrylamide induced rats, while significant increase comparing to normal control rat recorded 11.57, 10.72, 10.85 and 9.82 units, respectively.

In summary, the percentages of tailed, total, tail moment of DNA as well as its length in acrylamide intoxicated rats showed higher percentages than control group, while the un-tailed DNA showed lower value which indicated low DNA degradation. Treatment with the selected nanoparticles improved these indices by variable percentages (Table 6 and Figure 1).

**Table 6: Therapeutic effects of SOAE, hesperetin and narnginen loaded with silicate nanoparticles on DNA degradation indices in acrylamide intoxicated rats**

| Groups | % Tailed DNA | % Un tailed DNA | % Total DNA | Tail Moment |
|---|---|---|---|---|
| -ve Control | 5.00 ± 0.02$^{a}$ | 95.00 ± 3.80$^{a}$ | 2.08 ± 0.01$^{a}$ | 5.30 ± 0.52$^{a}$ |
| SOAE nor. | 6.00 ± 0.05$^{a}$ | 94.00 ± 4.90$^{a}$ | 2.03 ± 0.03$^{a}$ | 4.34 ± 0.32$^{a}$ |
| Hesp. nor. | 4.00 ± 0.04$^{a}$ | 96.00 ± 5.00$^{a}$ | 2.46 ± 0.05$^{a}$ | 5.39 ± 0.12$^{a}$ |
| Nar.nor. | 5.00 ± 0.01$^{a}$ | 95.00 ± 4.55$^{a}$ | 2.28 ± 0.04$^{a}$ | 5.72 ± 0.51$^{a}$ |
| Acrylamide | 18.00 ± 1.00$^{b}$ | 82.00 ± 6.10$^{b}$ | 5.02 ± 0.05$^{b}$ | 24.50 ± 0.23$^{b}$ |
| SOAE +ve | 14.00 ± 0.95$^{c}$ | 86.00 ± 5.90$^{c}$ | 3.66 ± 0.03$^{c}$ | 11.57 ± 0.15$^{c}$ |
| Hesp. +ve | 15.00 ± 0.88$^{c}$ | 85.00 ± 4.22$^{c}$ | 3.36 ± 0.01$^{c}$ | 10.72 ± 0.25$^{c}$ |
| Nar. +ve | 12.00 ± 0.96$^{d}$ | 88.00 ± 3.10$^{d}$ | 3.09 ± 0.02$^{c}$ | 10.85 ± 0.31$^{c}$ |
| Ref. Drug | 11.00 ± 0.10$^{d}$ | 89.00 ± 6.22$^{d}$ | 3.22 ± 0.05$^{c}$ | 9.82 ± 0.33$^{c}$ |

Values are % of the total counts in each assay. Each parameter was done in triplicate. Tail Moment (unit)=Tail length × % total DNA. Data are mean percentage ± SD of six rats in each group statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version 8), where unshared letters between groups are the significance value at $p \leq 0.05$

*Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on on ATPase enzyme activity and total protein content in brain tissue of acrylamide induced rats*

In addition nanoparticles of SOAE and naringenin administered to healthy rats showed an insignificant increase in ATPase enzyme activity and total protein content. As compared to normal healthy rats, acrylamide intoxicated rats showed significant decrease in ATPase enzyme activity and total protein content by 27.00% and 67.30%, respectively. Moreover, insignificant change was recorded in ATPase activity in acrylamide induced rats upon treated with the selected nanoparticles as well as standard drug. Concerning total protein content, it showed significant increase as compared to intoxicated rats, while significant decrease comparing to normal rats with percentages of amelioration 51.00%, 50.00% and 51.71%, respectively upon treated acrylamide induced rats with SOAE, hesperetin and standard drug. While, naringenin declared insignificant change.

With respect to, the recorded change in ATPase enzyme activity, naringenin nanoparticle administered post acrylamide showed improvement percentage by 23.20%, followed by SOAE nanoparticle (22.30%). Also, naringenin nanoparticle administered post acrylamide showed the highest improvement percentage (66.61%), in total protein content followed by donepezil (51.71%), and SOAE( 51.30%) (Table 7).

## DISCUSSION

The main toxic endpoints of ACR are known as neurotoxicity in humans and animals, developmental and reproductive toxicity in rodents, and nontoxicity and carcinogenicity in rodents. Neurotoxicity of ACR is known from accidental intoxications and from

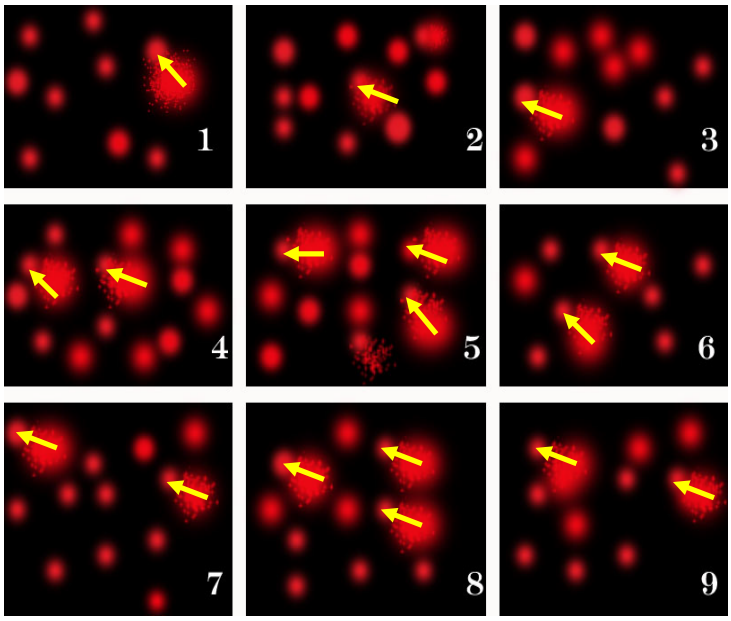


**Figure 1: DNA degradation pattern in brain cell of acrylamide intoxicated rats after treatment with SOAE, hesperetin and naringenin nanoparticles as compared to control group (Comet technique). 1-negative control, 2-hesperetin nanoparticle 3-naringenin nanoparticle, 4-SOAE nanoparticle, 5-acrylamide intoxicated rats, 6-hesperetin nanoparticle administered post acrylamide treatment, 7-naringenin nanoparticle administered post acrylamide treatment, 8-SOAE nanoparticle administered post acrylamide treatment, 9-donepezil HCl administered post acrylamide**

**Table 7: Effect of SOAE, hesperetin and naringenin loaded with silicate nanoparticles on ATPase enzyme activity and total protein content in brain tissue of acrylamide induced rats**

| Groups / Parameters | Control (-ve) | Treated controls | | | Acrylamide (+ve) | Acrylamide treated | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SOAE | Hesperetin | Naringenin | | SOAE | Hesperetin | Naringenin | donepezil Hcl |
| ATPase Mean | 8.52 ± 0.41$^{a}$ | 7.92 ± 0.71$^{abc}$ | 7.80 ± 0.29$^{bc}$ | 8.37 ± 0.41$^{ab}$ | 6.22 ± 0.22$^{d}$ | 8.12 ± 0.46$^{abc}$ | 7.60 ± 0.21$^{a}$ | 8.20 ± 0.54$^{abc}$ | 7.97 ± 0.76$^{abc}$ |
| % Change compared to control rats | | 7.00 | 8.51 | 1.80 | 27.00 | 4.70 | 10.81 | 3.81 | 7.31 |
| % Change compared to intoxicated rats | | | | | | 30.55 | 22.19 | 31.83 | 28.14 |
| % of Improvement | | | | | | 22.30 | 16.22 | 23.20 | 20.50 |
| Total protein Mean | 74.15 ± 2.04$^{a}$ | 71.92 ± 1.96$^{a}$ | 71.32 ± 1.56$^{a}$ | 71.12 ± 1.67$^{a}$ | 24.27 ± 3.02$^{c}$ | 62.28 ± 2.03$^{b}$ | 68.45 ± 2.83$^{d}$ | 73.65 ± 2.31$^{a}$ | 62.62 ± 2.30$^{b}$ |
| % Change compared to control rats | | 3.00 | 3.81 | 4.08 | 67.32 | 16.00 | 17.62 | 0.70 | 15.50 |
| % Change compared to intoxicated rats | | | | | | 156.61 | 151.83 | 203.46 | 158.01 |
| % of Improvement | | | | | | 51.30 | 50.00 | 66.61 | 51.71 |

Data are means ± SD of six rats in each group. Data are expressed as umoles /g wet weight for ATPase enzyme while total protein is expressed as mg/gram of brain tissue. Statistical analysis is carried out using SPSS computer program coupled with Co-Stat computer program (version 8), where unshared letters between groups are the significance value at $p \leq 0.05$

chronic occupational exposures [38]. The present results clearly demonstrate significant reduction in brain neurotransmitter levels (adrenaline, noradrenaline, serotonine and dopamine), ATPase enzyme activity and total protein content while significant elevation in acetylcholine esterase enzyme activity, DNA damage and caspase-3 enzyme activity in ACR intoxicated rats. A body of evidence suggests that oxidative stress mechanisms plays a vital role in ACR-induced neurotoxicity [39]. ACR is known to affect membranes and cytoskeletal protein via oxidative stress resulting in disrupted neurotransmission in rodents [40,41]. Goldstein and Lowndes [42] suggested that defective neurotransmission in ACR-intoxicated laboratory animal might be mediated by changes in transmitter synthesis, storage uptake and release [42,43]. In concomitant with the present results LoPachin et al. [44], showed that significant decrease in epinephrine, norepinephrine and dopamine contents in all tested brain areas at higher dose of acrylamide; this may be due to; axon and nerve terminal degeneration which caused changes in transmitter synthesis, storage uptake, release and reduction

in synaptic vesicle as a result the content of neurotransmitters is decreased. With ACR exposure, dopaminergic neurotransmission appears to be largely affected as evident by significant depletion in DA levels. Previous workers have demonstrated that ACR reduces DA levels in brain and furthermore affects DA receptor density, DA release and uptake both *in vivo* and *in vitro* exposure [45]. ACR affects both cholinergic and dopaminergic neurotransmission [46]. It is well accepted that energy depletion and mitochondrial dysfunctions apart from oxidative stress are vital factors associated with most of the neurodegenerative mechanisms [47]. In the present study, ACR markedly enhanced the activity levels of AchE, a significant biological component of the cholinergic function and membrane. AchE is known to contribute to membrane integrity and permeability occurring during synaptic transmission and conduction [48]. Enhanced activation of AchE leads to faster acetylcholine degradation and a subsequent down stimulation of acetylcholine receptors which causes a reduction of cholinergic neurotransmission and related functions such as cell proliferation and promotes apoptosis [49]. The Monoamine neuro-transmitter serotonin (5-HT) is concentrated in a cluster of neurons in the region of brain stem, involved in temperature regulation, learning, sensory perception and onset of sleep [50]. It is known that transmission of nerve impulse involves several steps, including transmitter synthesis, storage, release, reaction with receptor and termination of transmitter actions [51]. Evidence has been adduced to show that ACR interacts with tyrosine and tryptophan. Tyrosine is the precursor amino acid for synthesis of catecholamine's such as DA and NA. It is likely that by complexing with tyrosine, ACR may decrease the critical concentration of tyrosine in brain leading to an inhibition of catecholamine synthesis. Reactivity of ACR with tryptophan may presumably result in an inhibition of biosynthesis f 5-HT which requires precursor amino acid, tryptophan [52]. The current results clearly demonstrated significant increase in caspase enzyme activity in brain tissue of acrylamide intoxicated rats. The current results run in parallel with Sumathi et al. [53] demonstrated acrylamide toxicity preceded an elevation in DNA destruction as specified by the DNA fragmentation increment as well as the number of comets recorded. Fragmentation of DNA beside the elevation in the Comets appearance has also been declared by Bondy et al. [54] as a consequence of exposure to acrylamide. Further, acrylamide is recognized to enhance a reactive oxygen species level which is renowned, to elicit destruction for various macromolecules as well as to DNA. DNA injury is considered to be one of the biomarkers and identical discriminatory of apoptosis [53]. Hence, the current study markedly investigated that, the toxicity in response to acrylamide can enhance apoptosis as represented in the comet micrographs which obviously detected cells derangement.

In addition the current research recorded significant reduction in the activity of ATPase in acrylamide intoxicated rats. This result is in concomitant with Martyniuk et al. [55] and Sumathi et al. [53] who found that, ACR was shown to influence energy output by preventing glycolytic pathway, so the low activity of ATPase would in turn particularly modified the ion exchange and cells neurotransmission.

It is considered that ATPase be one of the principal ingredient, of the ACR induced -neurotoxic effect which leads to distal and recessive nerve fiber degradation [12]. Moreover, accumulation of Aβ as a consequence of acrylamide exposure causes hydrogen peroxide and hydroxyl radical production via specific chemical reactions. The output of these reactive oxygen species stimulated membrane lipid peroxidation, which can deteriorate the membrane function ion-motive ATPase (Na+/K+- and Ca+-ATPases) leading to depolarization of membrane as well as a reduction in ATP cellular levels [45].

With respect to total protein content, the current results demonstrated significant decrease in total protein content in the ACR induced rats. This observation is in accordance with the results of several authors [56,57], as they found that, brain toxicity is accompanied by a fall in whole-body protein turnover. The reduction in the total protein content in the brain injected with ACR may be due to the increase in amino acids deamination and impairment in cellular proteins construction. Sherlock and Dooley [58], ascertained perturbation in protein synthetic machinery in the brain. Furthermore, the lower protein level that is observed in ACR induced rats might be also related to formation of the toxic N-nitroso compounds lead to suppressing of oxidative phosphorylation as mentioned by Anthony. Also, the Nitroso-compounds interact with cellular DNA, RNA and protein resulted in biochemical and physical alterations of these macromolecules [59]. Flavonoids are a group of naturally occurring substances, including flavones, flavanones, and isoflavones, having several beneficial biological activities of flavonoids, including antioxidant, antitumor, and anti-inflammation properties [60,61]. Some of these flavonoids (SOAE, hesperetin and naringenin), due to their phenolic structures, have antioxidant effect and inhibit free radical-mediated processes [62]. Naringenin was found to possess antitumor, anti-inflammatory and hepatoprotective effects [63]. In addition Hsp has been shown to be a potential anti-oxidant, anti-inflammatory, neuroprotective agent [64,65]. Accordingly, nano-components (SOAE, hesperetin and naringenin) which possess antioxidant properties are possibly to be defensive against ACR-induced neurotoxic effects [66]. In summary, the present data indicated that acrylamide induced brain damage which might be related to oxidative stress. Administration of the three nano-components lessened the negative effects of acrylamide on the brain by inhibiting free radical mediated process; an effect that could be attributed to the antioxidant property of three nano-components.

## REFRENCES

[1] L. Camachoa, J.R. Latendresseb, L. Muskhelishvilib, R. Pattonb, J.F. Bowyerc, M. Thomasc, D.R. Doerge, *Toxicol. Lett*., **2012**, 211, 135-143

[2] A. Becalski, B.P.Y. Lau, D. Lewis, S.W. Seaman, *J. Agr. Food. Chem*., **2003**, 51, 808-8021.

[3] R.G. Tardiff, M.L. Gargas, C.R. Kirman, M.L. Carson, L.M. Sweeney, *Food Chem. Toxicol*., **2010,** 48, 658-667.

[4] World Health Organization (WHO), **2005**.

[5] C.J. Calleman, Y. Wu, F. He, G. Tian, E. Bergmark, S. Zhang, H. Deng, Y. Wang, K.M. Crofton, T. Fennell, *Toxicol. Appl. Pharmacol*., **1994**, 126, 361-371.

[6] G.E. Johnson, S.H. Doak, S.M. Griffiths, E.L. Quick, D.O. Skibinski, Z.M. Zair, G.J. Jenkins, *Mutat. Res.,* **2009**, 678, 95-100.

[7] M.A. Friedman, E. Zeiger, D.E. Marroni, D.W. Sickles, *J. Agric. Food Chem*., **2008**, 56, 6024-6030.

[8] R. Lucchini, L. Benedetti, E. Albini, L. Alessio, *Int. Arch. Occup. Environ. Health*., **2005**, 78, 427-437.

[9] Research and Consultancy Solutions (RNCOS), **2007**.

[10] D.S. Rohlman, R. Lucchini, W.K. Anger, D.C. Bellinger, C. Van Thriel, *Neurotoxicol*., **2008**, 556-567.

[11] R.M. LoPachin, *Neurotoxicol*., **2003**, 25, 617-630.

[12] R.M. LoPachin, C.D. Balaban, J.F. Ross, *Toxicol. Appl. Pharmacol*., **2004**, 188, 135-153.

[13] E.J. Lehning, R.M. Lopachin, J. Matthew, J. Eichberg, *J. Toxicol. Environ. Health*., **1994**, 42, 331-342.

[14] M.I. Yousef, F.M. El-Demerdash, *Toxicol*., **2006**, 133, 219-214.

[15] G. Oberdörster, E. Oberdörster, J. Oberdörster, *Environ. Health. Perspect*., **2005**, 113, 823-839.

[16] L.L. Hsieh, H.J. Kang, H.L. Shyu, C. Chang, *Water. Sci. Technol*., **2009**, 60, 1295-1301.

[17] J.J. Wang, B.J. Sanderson, H. Wang, *Mutation res*., **2007**, 628, 99-106.

[18] Y.Chen, S.C. Shen, H.Y. Lin, *Biochem. Pharm*., **2003**, 66, 1139-1150.

[19] S.L. Hwang, G.C. Yen, *J. Agric. Food Chem*., **2009**, 57, 2576-2582.

[20] M.J. Jeff, Therapeutic Research Facility. Natural Medicines Comprehensive Database, 4th edn, **2002**.

Y, [21] L.T. Yi, C.F. Li, X. Zhan, C.C. Cui, F. Xiao, L.P. Zhou, Y. Xie, Involvement Prog. *Biol. Psychiatr*., **2010,** 34, 1223-1228.

[22] A. Russo, R. Acquaviva, A. Campisi, V.Sorrenti, C. Di Giacomo, G. Virgat, M.L. Barcellon A.Vanella, *Cell Biol. Toxicol*., **2000**, 16, 91-98

[23] I. Erlund, M.L. Silaste, G. Alfthan, M. Rantala, Y.A. Kesaniemi, A. Aro, *Eur. J. Clin. Nutr*., **2002**, 56, 891-898.

[24] E.J. Choi, W.S. Ahn, *Arch. Pharm. Res*., **2008**, 31, 1457-1462.

[25] S.H. Jeon, W. Chun, Y.J. Choi, Y.S. Kwon, *Arch. Pharm. Res*., **2008**, 31, 978-982.

[26] J.M. Vasconcelos, A.M.S. Silva, J.A.S Cavaleiro, *Phytochem*., **1998**, 49, 1421-1424.

[27] A.A Haroun, A.M. Elnahrawy, H.I. Abd-Alla, **2016**.

[28] K. Pradeep, S. Park, K. CheolKo, *European J. Pharmacol.,* **2008**, 587, 273-280

[29] A. Jain, A. Yadav, A.I. Bozhkov, V.I. Padalko, S.J.S. Flora, *Ecotoxicol. Environ. Saf.,* **2011**, 74, 607-614.

[30] S. Sonkusare, K. Srinivasan, C. Kaul, P. Ramarao, *Life Sci*., **2005**, 77, 1-14.

[31] H.A. Fernando, *Diabetes. Res*., **2013**, 95, 2013:1903.

[32] J. Zagrodzka, A. Romaniuk, M.Wieczorek, P. Boguszewski, *Acta. Neurobiol. Exp*., **2000**, 60, 333-343.

[33] G. Wen, W. Hui, C. Dan, W. Xiao-Qiong, T. Jian-Bin, L. Chang-Qi, L. De-Liang, C. Wei-Jun, L. Zhi-Yuan, L. Xue-Gang, *Acta. Histochem. Cytochem.*, **2009**, 42, 137-142.

[34] B. Savitha, C.N. Naik, R. Guruprasad, S. Arjunp, S. Priyanka, S. Math, *J. Inves. Clini.,* **2014**, Dentistry.

[35] N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, *Exp. Cell. Res*., **1988**, 175, 184-191.

[36] E. Matteucci, F. Cocci, L. Pellegrini, G. Gregori, O. Giampietro, *Enz. Protein*., **1994**, 48(2), 105-119.

[37] M.M. Bradford, *Anal. Biochem*., **1976**, 72, 248-254.

[38] P. Wolfram, *Food. Chem. Toxicol*., **2008**, 46, 1360-1364.

[39] S. Mehri, K. Abnous, S.H. Mousavi, V.M. Shariaty, H. Hosseinzaddeh, *Cell. Mol. Neurobiol*., **2011**, 30, 185-191.

[40] S. Yu, F. Son, J. Yu, X. Zhao, L. Yu, G. Li, *Neurochem. Res*., **2006**, 31, 1197-2

[41] Y.J. Zhu, T. Zeng, Y.B. Zhu, S.F. Yu, Q.S. Wang, L.P. Zhang, *Neurochem. Res*., **2008**, 33, 2310-2317.

[42] B.D. Goldstein, H.E. Lowndes, *Neurotoxicol*., **1986**, 2, 297-312.

[43] D.W. Sickls, S.T. Brady, A. Testino, M.A. Friedmanand R.W. Wrenn, *J. Neurosci. Rev*., **1996**, 46, 7-17.

[44] R. LoPachin, D. Barber, D. He, S. Das, *Toxicological. Sci*., **2006**, 89(1), 224-234.

[45] H.H. Ahmed, G.A. Elmegeed, S.M. El-Sayeed, M.M. Abd-Elhalim, W. ShoushaGh, R.W. Shafic, *Eur. J. Med. Chem*., **2010**, 45, 5452-5459.

[46] B. Ling, N. Authier, D. Balayssac, A. Eschalier, F. Coudore, **2005**, *Pain*., 119, 104-112.

[47] M.T. Lin, M.F. Beal, **2006**, *Nature*., 443, 787-795.

[48] R. Schmatz, C.M. Mazzanti, R. Spanevello, N. Stefanello, J. Gutierres, M. Correa, *Eur. J. Pharmacol*., **2009**, 610, 8-42.

[49] Q.H. Jin, H.Y. He, Y.F. Shi, X.J. Ahang, *Acta. Pharmacol*., **2004**, 25, 1013-1021.

[50] D.S. Strac, D. Muck-Seler, N. Pivac, *Psychiatria. Danubina*., **2015**, 27(1), 14-24

[51] N.S. Prasad, Muralidhara, *Neuro. Toxicol*., **2012**, 33, 1254-1264.

[52] M.E. Hidalgo, C. De la Rosa, *Quim Nova*., **2009**, 70, 32-1467.

[53] T. Sumathi, C. Shobana, V. Mahalakshmi, R. Sureka, M. Subathra, A. Vishali, K. Rekha, *Asian. J. Pharm. Clin. Res*., **2013**, 6, (3) 80-90.

[54] S.C. Bondy, D. Liu, S. Guo-Ross, *Neurochem. Int*., **1998**, 33, 51-54.

[55] C.J. Martyniuk, B. Fang, J.M. Koomen, T. Gavin, L. Zhang, D.S. Barbert, *Chem. Res. Toxicol*., **2011**, 24, 2302-2311.

[56] L. Wang, X. Yan, Z. Zeng, J. Lv, P. Liu, C. Liu, **2010**, 35, 1740-1744.

[57] J. George, K.R. Rao, R. Stern, G. Chandrakasan, *Toxicol.*, **2001**, 156, 129-138.

[58] S. Sherlock, J. Dooley, Malden, MA: Blackwell Science **2002**.
[59] M.L. Anthony, K.P. Gartland, C.R. Beddell J.K. Lindon, *Arch.Topical*., **1994**, 68, 43-53.
[60] G. Ramakrishnan, H.R. Raghavendran, R. Vinodhkumar, T. Devaki, *Chem. Biol. Interact*., **2006**, 161, 104-114.
[61] Y.C. Chen, S.C. Shen, W.R. Lee, W.C. Hou, L.L. Yang, T.J.F. Lee, *J. Cell. Biochem*., **2001**, 82, 537-548.
[62] Y.C. Chen, S.C. Shen, W.R. Lee, H.Y. Lin, C.H. Ko, C.M. Shih, L.L. Yang, *Arch. Toxicol*., **2002**, 76, 351-359.
[63] N.J. Montvale, PDR for Herbal Medicines. *Medical Economics Company*., **2000**.
[64] A. Hirata, Y. Murakami, M. Shoji, Y. Kadoma, S. Fujisawa, *Anticancer. Res.,* **2005**, 25, 3367- 3374.
[65] E.J. Choi, *Life. Sci*., **2008**, 82, 1059-1064.
[66] Q. Xie, Y. Liu, H. Sun, Y. Liu, X. Ding, D. Fu, *J. Agr. Food. Chem*., **2008**, 56, 6054-6060.