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Research Article
Garden Cress Seed Extract in Natural and Nano Forms to Inhibit Rat's Oxidative Stress

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Abstract

Background and Objective: Garden cress (*Lepidium sativum* L.) is a fast growing annual plant, the garden cress seed is the herbal edible seeds used in traditional medicine. A number of experimental have been conducted on rats that proven the efficacy of garden cress seeds (GCS) and its protective role in many diseases. Also, the GCS was used in the fortification of different food items. In this study focusing on the potent activity of polyphenol in the natural GCS extract or nanoemulsion of GCS extracts to reduce the oxidative stress in rat induction by paracetamol (PCM) and determined of the potentiality of the two GCS extracts which to be limited. **Materials and Methods:** The current study evaluated the polyphenol content of the GCS estimated by HPLC *in vitro* and *in vivo* determined polyphenols effects in a healthy Wister male rats (32 rats weighing 130±15) which divided into 4 groups to examine antioxidant activity of 2 extracts (Natural and nano) forms. In addition, the best average size of nanoparticles was 198.1±0.553 nm and 90% of the distribution determined by dynamic light scattering (DLS) instrument showed in A_1 sample nanoemulsion. **Results:** The findings showed that the natural GCS extract more potential than the nanoemulsion in the antioxidant activity, it may be related to destroyed some of active polyphenols exist naturally in the GCS extract during formulation GCS nanoemulsion by sonication process. Utilizing PCM in over dose was produced N-acetyl-p-benzoquinone imine (NAPQI) compound which exceeded the liver rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of proteins and alters the homeostasis of calcium after depleting GSH. **Conclusion:** This study proven that the intake of natural GCS extract as antioxidants is important with the administration PCM notably to avoid side effects of overdose of PCM.

Key words: Garden cress seeds (GCS), natural and nano-forms, oxidative stress, paracetamol, N-acetyl-p-benzoquinone imine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the last three decades there has been more interest in understanding the role of antioxidants in treatment of many diseases such as cancer, arteriosclerosis, aging and many compounds induce oxidative stress and usual cause severe diseases. The oxidative process play main role in numbers of human neurologic disorders and other illnesses[1]: for example, oxidative stress was increased in diabetes which parallel with reduction in the antioxidant status and make defect in the many chronic complications of diabetes[2,3]. In the same time carcinogenesis agents mechanisms mainly the reactive oxygen species are play role to incidence many types of cancer which the mechanism start with DNA destroyed and occur of genetic mutation in one or few cell lines to turned to dysplastic cellular line, release cell growth and in the end carcinoma[4].

Medicinal plants have a potent protective role and gained great attentions to be used as natural food additives as condiment in food stuff[5]. GCS is affined to Brassicaceae and named by many trade name as "Habel Rashaad" or "Thufa" in Middle East countries[6]. The GCS are used in traditional medicine applications and the leaves of GCS are used in salad[7]. Many studies investigated the GCS protective effects as anti-carcinogenic[8], antidiabetic[9], anti-asthmatic, diuretic[10], relief hypotensive disease[11] and antibacterial agent[12]. The most active ingredient content in GCS that isothiocyanates, which is formative with glucosinolates determined as well as the high inducers to detoxifying enzymes and reduce carcinogen agents. The active contents of isothiocyanates are benzyl isothiocyanate (BITC), it is abundant ingredient exist plenty quantity in GCS[13]. The seeds mainly acquired many properties as bitter, are galactogogue, depurative, rubefacient, aphrodisiac, ophthalmic, antihistaminic, diuretic and act as tonic. In addition, it treat various diseases such as dysentery, skin disease, coughs with expectoration, asthma, poultices for sprains, leprosy, splenomegaly, dyspepsia, leucorrhoea, scurvy, seminal weakness and others health illnesses[14].

PCM considered a quite safe drug at recommended doses which formative of para-aminophenol group and is ordinarily useful in humans as analgesic-antipyretic[15]. PCM (acetaminophen) generally used as drugs to relieve mild to moderate pain, as well as to reduce fever in most countries and worldwide, it is obtainable without a prescription of physician. PCM overdose usually induce hepatotoxicity also, many researchers demonstrated that PCM caused hepatotoxicity. In order to PCM triggers hepatotoxicity meanwhile usually induced nephrotoxicity. Renal defect is reported to occur in nearly (1-2%) of patients in tolerance to paracetamol toxicity[16]. After intake PCM dosages, about 63% oral administration is metabolized in the liver via many mechanisms such glucuronidation and 34% via sulphation primarily. Accordingly, compounds were water-soluble pathways are excreted by the 2 kidneys. The only intermediate kinetic mechanism produce N-acetyl-p-benzoquinone imine (NAPQI) is a reactive that occurs by the microsomal P-450 enzyme system when oxidization of 55% of PCM. The endogenous antioxidants enzyme such as intracellular glutathione (GSH) is detoxified NAPQI by therapeutic doses[17]. Also, oral intake PCM overdose implicated as responsible to produce intermediate metabolite NAPQ which has been a toxic compound[18].

Finally, the active compounds as antioxidants of natural sources are useful to protective many diseases. So, nowadays natural herb active substance attracted many previous studies. This natural compounds lead to more interest to demonstrate the effects of the GCS as a natural source of active ingredients. Thus, current study's main target was used the active natural ingredients of GCS notably antioxidants compounds in 2 forms. The first form is natural GCS extract and the second one nano-formulation of GCS extract to study sufficient role of antioxidant GCS extracts (natural and nano forms) and the protective effect *in vivo* as anti-oxidative stress which induced by PCM overdose.

MATERIALS AND METHODS

Study area: The experimental implemented in lab and animal house of the National research Centre, Cairo, Egypt in the duration between May and October, 2018.

Plant material: The plant was purchased from local market in Egypt. The plant was classified and authenticated as *Lepidium sativum* L. by Botanists in College of Agricultural Sciences, Cairo University. The plant seeds were ground as fine powder and the powder was kept in air tight container till Extract antioxidants solution.

Animals: Healthy wister male rats (32 rats) weighing 130±15 g were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt and maintained in clean plastic cages in the laboratory animal room (23±2°C). All rats food intake on standard pellet diet prepared formula according AIN-93 purified diets for laboratory rodents[19], tap water ad libitum and daily dark/light cycle (12/12 h). The rats were acclimatized for 1 week prior to the start of experiments. The experimental work on rats was performed with the approval of the Animal Care and

Experimental Committee, National Research Centre, Cairo, Egypt and international guidelines for care and use of laboratory animals[20].

Chemicals: The kits used for biochemical measurements of malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), transaminases (ALT and AST), alkaline phosphatase (ALP), albumin, creatinine and urea were all purchased from Biodiagnostic Company, 29 Tahreer St., Dokki, Giza, Egypt. All other chemicals were of reagent grades and were obtained from the local scientific distributors in Egypt.

Antioxidant GCS seed extraction: GCS (250 g), were purchased from a local market in Giza, Egypt, in October 2018. GC seeds crushed and socketed in 0.5 L of ethanol purity 96%. The cold socking continued until no more condensing oil could be considered (2 days). The antioxidant extract was separated and filtered, dried by KNF rotary evaporator RC 600 Germany to 50 mL, transferred to an amber glass flask and kept undercooled until use.

Nanoemulsion preparation: Antioxidant GCS extract nanoemulsion of seeds (10%) was prepared by using Tween-40 as a non-ionic surfactant. GCS extract and deionized water as mixed in glass flask. After that the organic phase was prepared by adding antioxidant GCS extract and Tween-40 in different ratios 1:0.5, 1:1 and 1:1.5 (v/v). Then, aqueous phase (DW) was added to organic phase and subjected to sonication for 15, 25 and 35 min using Ultrasonic (Sonics and Materials, Inc., 53 Church Hill Rd., Newtown, CT, USA) with a probe diameter of 13 mm at a high frequency of 20 kHz and power output of 750 W. To reduce energy, ice was used for cooling during the sonication process and energy was given through sonicator probe.

Droplet size analysis: The average size and distribution were determined by dynamic light scattering (DLS) instrument Particle Sizing Systems (PSS, Santa Barbara, CA, USA) at 23°C, using the 632 nm line of a He-Ne laser as the incident light with angle 90. The nano-emulsion of 1:0.5, 1: 1 and 1:1.5 (v/v) by the sustainable ratio of antioxidant GCS extract (10%) and Tween 40 with varied period sonication time to found which to be stable with a lowest droplet size diameter.

HPLC conditions: HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using C18 column (4.6×250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate 1 mL min^{-1}. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A), 0-5 min (80% A), 5-8 min (40% A), 8-12 min (50% A), 12-14 min (80% A) and 14-16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µL for each of the sample solutions. The column temperature was maintained at 35°C.

Preparation paracetamol suspension: A PCM suspension was prepared in 1% carboxymethyl-cellulose in saline phosphate buffer and animals were administered 2g kg^{-1} to rat weight of this suspension orally by gastric tube (1 mL of suspension contains 0.4 g of paracetamol).

Experimental rats design: Rats were divided randomly into 4 groups with 8 rats in each group as follow:

- Group 1 feed on normal standard diet (no treatment)
- Group 2 was ingestion PCM suspension 2 g kg^{-1} of rats weight during first 10th day of the experiment (induction period) by gavage
- Group 3 rats administered oral natural extract of GCS+PCM suspension (2 g kg^{-1} b.wt.)
- Group 4 rats received an oral dose Nano-formulation of GCS extract+PCM suspension (2 g kg^{-1} b.wt.)

After completion of treatment period (28 days), blood samples were withdrawn from the animals under light ether anesthesia by heart puncturing of the animals with a fine sterilized glass capillary tube. Blood samples were collected, subjected to serum separation and stored at -20°C for biochemical analysis within 1 week.

Rats were then killed by decapitation. Livers were immediately isolated, cleaned and kept in 10% of formalin solution for histological studies.

Liver and kidney function tests: Serum transaminases (ALT and AST) activities were determined by a colorimetric method according to Reitman and Frankel[21]. Serum alkaline phosphatase (ALP) activity and albumin were determined by enzymatic colorimetric method according to Young *et al.*[22].

Oxidative stress parameter:

- Malondialdehyde (MDA) content as indicator of lipid peroxidation was determined in the serum, by a colorimetric method according to Satoh[23]

- Reduced form of glutathione (GSH) was determined by colorimetric method according to Beutler *et al.*[24]

Liver antioxidant enzymes: Catalase (CAT) activity was measured according to the method described by Aebi[25] by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25°C 39. The activity of CAT enzyme is expressed as μ mL^{-1} protein.

Superoxide dismutase (SOD) activity was determined according to the method described by Marklund and Marklund[26] by assaying the autoxidation and illumination of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autoxidation inhibition. The SOD activity is expressed as U mL^{-1} protein.

Glutathione peroxidase (GPx) activity was measured using H_2O_2 as substrate according to the method described by Paglia and Valentine[27]. The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. Enzyme activity was expressed as μ mL^{-1} protein.

Statistical analysis: All studied data were statistically analyzed using Co-Stat 6.303 Software Computer Program 2004 hypothesis testing methods included one way analysis of variance (ANOVA) using dancnn test[28].

RESULTS

Effect of Tween 40 ratio and sonication time on formulation nanoemulsion: In the first Fig. 1(a-c), showed that the best ratio of surfactant to be used for antioxidant GCS extract nanoemulsion. The nanoemulsion was prepared in varied ratios of un-ionic surfactant (Tween 40) 1:0.5, 1:1 and 1:1.5 (v/v) respectively. Also, sonication times altered during sample prepared to 15, 25 and 35 min, by Ultrasonic to form nanoemulsion. Nanoemulsions of 1:0.5, 1:1 and 1:1.5 (v/v) ratios of antioxidant extract and Tween 40 with 35 min sonication times were observed from Fig. 1a to be stable as well as droplet size distribution was measured for these nanoemulsions. The measure scale of nanoemulsion which means droplet size diameter average was 198.1±0.553 nm and 90% of the distribution less than 284.4 nm, while the mean diameter of Fig. 1b nanoemulsion was 676±404 nm and 75% of the distribution less than 953 nm. So, the finding determined that the best nanoemulsion prepared formula (distribution and nanoparticles size) in Fig. 1c.

HPLC assessed polyphenol antioxidants in GCS extract natural and nano forms: The Fig. 2(a-c) determined polyphenols antioxidants in the natural GCS extract and GCS extract nanoemulsion, the findings clearly showed that HPLC chart beaks of the polyphenols decline in nano form more than in natural form of GCS extracts. In the same time, Table 1 confirmed that the high polyphenol content of gallic acid in natural GCS sample value 83.63 μg/0.5 mL and reduced in nanoemulsion of GCS extract to 46.13 μg/0.5 mL, respectively. In the same pattern other polyphenol contents declined

Table 1: Polyphenol antioxidants in GCS extract natural and nanoemulsion

Antioxidants	Natural GCS extract (μg/0.5 mL)	Nano-GCS extract (μg/0.5 mL)
Gallic acid	83.63	46.13
Catechin	16.90	2.45
Caffeine	18.88	0.47
Naringenin	42.08	1.47

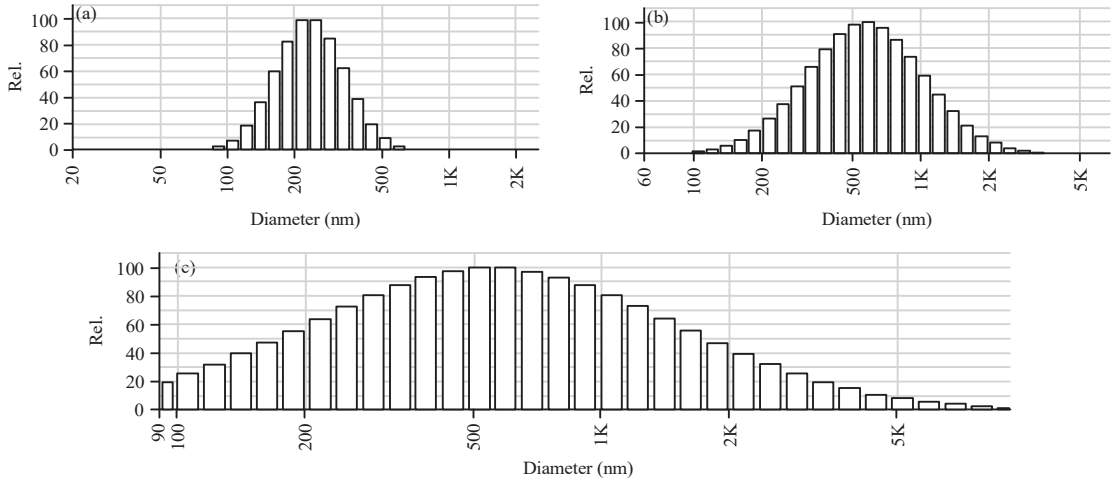

Fig. 1(a-c): Intens weight gaussian distribution, (a) Standard, (b) Antioxidant and (c) Nanoemulsion polyphenol

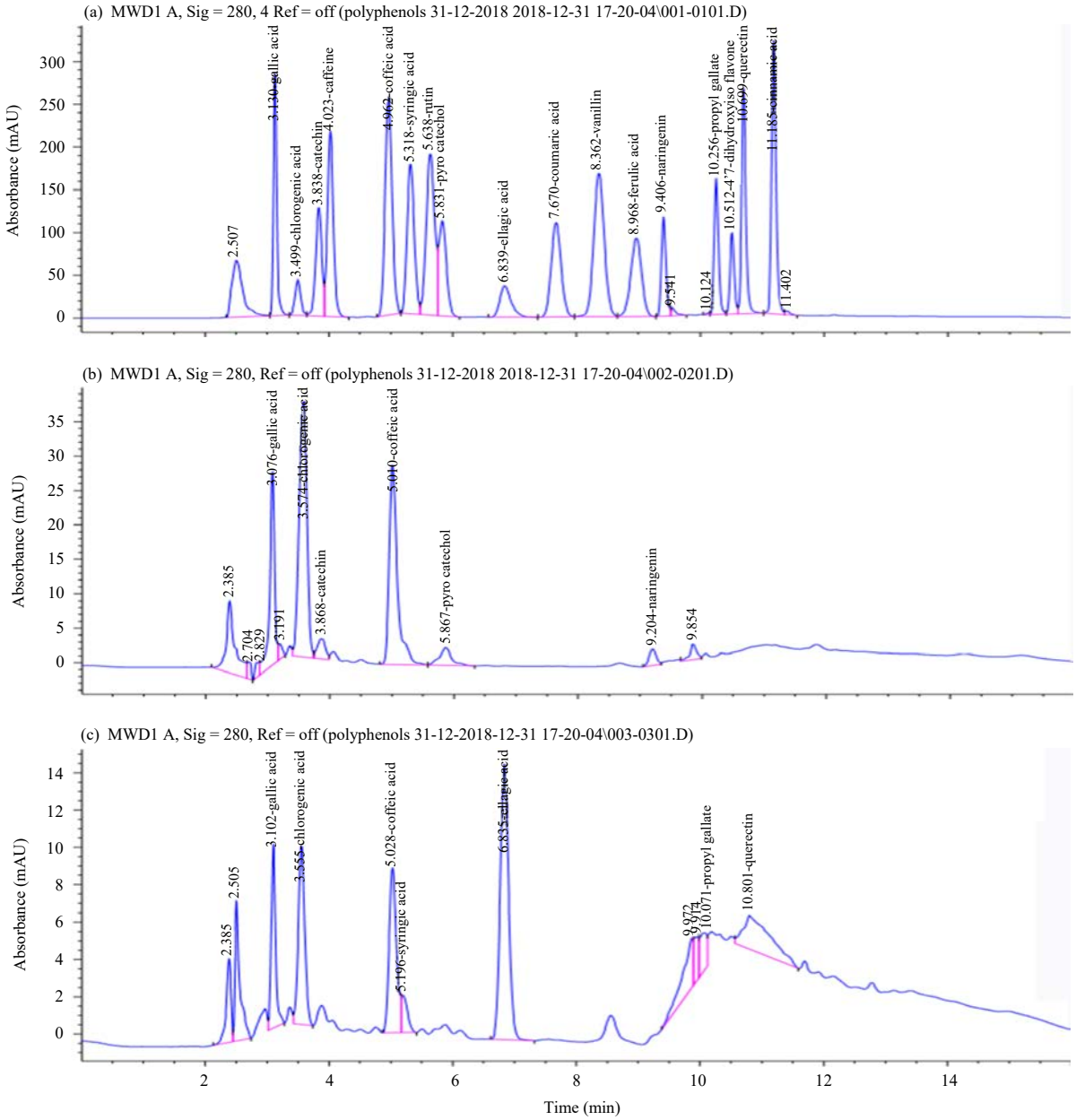

Fig. 2(a-c): Chromatogram of HPLC analysis of (a) Standards polyphenol beaks, (b) Antioxidant GCS extract polyphenol beaks and (c) Nanoemulsion GCS extract polyphenol beaks

in antioxidant nanoemulsion GCS extract than natural extract form. The Caffeine the lowest content in GCS nanoemulsion value 0.4 µg/0.5 mL and the Catechin polyphenol content in the natural GCS extract was 16.90 µg/0.5 mL, respectively.

Evaluation of the liver and kidney function in experimental rats: The results in Table 2 cleared the liver and kidney function nearly normal in rats after administration natural and nanoemulsion polyphenol antioxidant content of GCS extracts in groups 3 and 4. While in group 2 (positive control) was elevated in liver parameters AST, ALT and alkaline phosphatase value 59.63±6.44, 54.39±5.01 and 141.01±18.79 µ mL^{-1}, respectively. In addition, the urea was high significant values relationship with normal control and normal range but creatinine was slightly high in group 2 to 1.16 mg dL^{-1}. The oxidative stress of PCM overdose oral administration by rats, which the PCM swallowed gavage increased oxidation in liver enzymes as illustrated in Table 2.

Table 2: Assessment of liver and kidney function and administration of GCS extracts in rats

	Parameters					
Groups	AST (μ mL^{-1})	ALT (μ mL^{-1})	Alkaline phosphatase (μ mL^{-1})	Albumin (g dL^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
1	31.50±5.63^{ab}	30.63±5.85^{b}	112.13±11.18^{b}	4.26±0.66^{a}	38.53±6.83^{a}	0.74±0.14^{b}
2	59.63±6.44^{a}	54.39±5.01^{a}	141.01±18.79^{a}	3.95±0.52^{b}	39.98±8.19^{a}	1.16±0.20^{a}
3	41.80±4.16^{b}	39.55±3.34^{b}	119.75±6.84^{ab}	4.52±0.62^{a}	38.35±11.04^{a}	0.96±0.18^{b}
4	48.80±4.36^{b}	45.75±3.34^{b}	121.55±6.74^{ab}	4.30±0.42^{a}	35.23±10.04^{a}	0.99±0.28^{b}

^{ab}Means±SD in each column having the same letter were not significantly different, values with different superscript letters are significantly different at p<0.05

Table 3: Oxidative stress status with administration of GCS extracts in rats and antioxidant enzyme

	Parameters				
Groups	SOD (μ mL^{-1})	GPx (μ mL^{-1})	GSH (mg dL^{-1})	Catalase (U mL^{-1})	MDA (mmol mL^{-1})
1	40.88±7.45^{a}	171.65±12.62^{a}	48.85±9.18^{a}	625.15±9.52^{a}	167.64±12.73^{c}
2	21.13±8.48^{c}	129.39±14.529^{c}	24.47±8.22^{c}	36.26±11.25^{c}	221.38±27.35^{a}
3	32.38±5.51^{b}	159.88±21.18^{b}	35.51±7.73^{b}	52.72±9.84^{b}	184.34±14.22^{b}
4	35.58±8.50^{b}	138.88±22.17^{b}	31.61±7.63^{b}	45.76±9.33^{b}	195.33±13.92^{b}

^{abc}Means±SD in each column having the same letter were not significantly different, values with different superscript letters are significantly different at p<0.05

Antioxidants activity *in vivo* of experimental rats: The activity of antioxidant enzyme superoxide dismutase (SOD), MDA and GSH and the PCM oxidative stress on rat liver showed in Table 3. The SOD activity level in groups 3 and 4 were found to be decreased to 32.38 and 35.58 μ mL^{-1}, Also the GSH reduced to 35.51 and 31.61 mmol mL^{-1} lower than normal group (1) respectively, with the consumption of GCS extracts forms. MDA measurement value was recorded to be significantly increased in the PCM oral overdose group (2) compared with the groups 3 and 4 (p<0.05) respectively. Indeed, the GCS antioxidants extract was ingested to rats gavage sustained GSH and SOD and decreased MDA levels in liver nearly as normal group I (Table 3).

DISCUSSION

No doubt, both 2 factors surfactant concentration and the time of sonication played an important role in formation of nanoemulsion stability. In this study on nanoemulsion which the ratio was 1:0.5 (v/v) of GCS extracts (10%) and Tween 40 with sonication period 35 min was noticed that nanoemulsion a lowest stable droplet size diameter (198.1±0.553 nm). So, this nanoemulsion (sample A1) was used for further evaluation as HPLC analysis of antioxidant GCS extract polyphenol content and proceeded *in vivo* studies on experimental Wister albino rats.

Nevertheless, GCS extract nanoemulsion evaluated effects in this current study regarding to GCS contains 20-25% of oil and the main fatty acid is linolenic acid (32-35%), GCS oil contains many active compounds chiefly unsaturated fatty acids, α-tocopherol and have several pharmacological properties like antibacterial, antifungal, antioxidant, anti-inflammatory. This oil is also responsible for recovery and relieving the pain in joints as rheumatism[29]. But as the findings amounted to polyphenol anti-oxidant contents decline in GCS extract nanoemulsion than the natural GCS extract which lead to slightly reduce in effects on oxidative stress was induced in albino rats by PCM induction. Moreover, the hepatotoxicity resulted to PCM metabolite residues compound has been attributed to the formation as active toxic compounds a part of PCM activated compound N-acetyl-P-benzoquinone imine (NAPQI). The detoxification process was cleaned by hepatic cytochrome p450 metabolites of N-acetyl-p-benzoquinone imine (NAPQI)[30,31].

In addition, exposing rat to PCM overdosing oral intake released oxidation of NAPQI free radical combined with glutathione decline by a high elevation in the concentration of NAPQI induced oxidation stress and incidence inflammation and may convert to necrosis[32]. In fact, these conditions conducted to produce a new treatment lead to suppress active compounds released reactive species of NAPQI as GCS extracts. Moreover, many drugs utilized clinically as N-acetyl-cysteine (NAC) for PCM overdose toxicity[33]. The NAC drug mechanism kinetic that increase cysteine to elevate the intracellular GSH level[34]. The overdose of PCM to be considered toxic and induce free radicals in an organ of the body such oxidative deleterious which plays role in damage hepatorenal by PCM overdose toxicity[35,36]. Therefore, natural active antioxidant compounds such as Gc antioxidant extract having activity could be used for natural alternative treatments of PCM toxicity. In spite of the results distinct that natural GCS extracted more potent useful than of GCS extract as well as antioxidant activity, it may be during nanoemulsion manipulate happened destroy in some of active polyphenol which exist naturally in the GCS extract by sonication process.

Really, this study on the experimental rat to assessment the liver and kidney function. Also, findings proven the

influence of GCS extract on hepatoprotective against hepatotoxic agents[37]. The natural GCS extract showed that inhibition effect on oxidative stress which oral dose to rats by gavage had significant higher scavenging activity effects on NAPQI hazard active compound to maintain liver and kidney in normal function. Meanwhile, may be the scavenging properties effect of the natural GCS extract of oral dose result to highly antioxidant activity content. Also, take in account the increased rate of formation NAPQI correlated with detoxification by GSH enzyme activity should exceed, in a state of exceed NAPQI compounds may oxidized tissue macromolecules such as lipid or -SH group of proteins and alters the homeostasis of calcium regard to injuries occurring in kidney after depleting GSH. This evidence cleared that the important role of the GCS extract as antioxidants should be ingested with the treatment by PCM oral dose notably in case of PCM overdose in natural GCS extract form.

CONCLUSION

The GCS contain natural antioxidants and bioactive agents to improve human health, but as this study findings of study amounted to polyphenol antioxidants contents decline in GCS extract nanoemulsion than the natural GCS extract which lead to slightly reduced in effectiveness on oxidative stress *in vivo* as well as in the case of induced oxidative stress in albino rats by PCM. Meanly the hepatotoxicity occurred when PCM metabolite in liver with attributed to the formation N-acetyl-p-benzoquinone imine (NAPQI).

SIGNIFICANCE STATEMENT

This study discovers the possible effectiveness of natural garden cress seed extract can be protect oxidative stress of consumption oral overdose of paracetamol in rats. This study will help the researcher to uncover the critical area of alternative natural herbal extract to chemical drugs used in overdose toxicity as N-acetyl-cysteine (NAC) that many researchers were not able to explore. Thus, a new trend as alternative medicine of these natural garden cress seed extract not nano emulsion extract of garden cress seed which to be less efficacy.

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