Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2020.0602.07**

EFFECT OF *Allanblackia floribunda* STEM BARK EXTRACT ON THE ANTIOXIDANT STATUS OF MALE WISTAR ALBINO RATS

Shimfe Q. H, Gabriel-Brisibe C.U*, John G.N.

Department of Medical Biochemistry, College of Medical Sciences,

Rivers State University, Nigeria.

ABSTRACT: This study investigated the subacute effects of the stem bark of *Allanblackia floribunda* on antioxidant status of male wistar albino rats. 28 male wistar rats of average weight between 115-140 g were obtained and distributed into four groups of seven rats each. Rats in the control group where administered Carboxyl Methyl Cellulose (CMC) while others where orally administered extract of *Allanblackia floribunda* at 100 mg/kg, 300 mg/kg and 600 mg/kg. On the 15th day, the animals were sacrificed and dissected and the liver was collected, homogenized, then centrifuged and supernatant was used for futher analyses. Malondialdehyde (MDA) was estimated for lipid peroxidation while Liver Superoxide Dismutase (SOD) and reduced Glutathione (GSH) were estimated for antioxidant status. There was a dose-dependent increase in MDA concentration. The activity of SOD increased with administration of A.floribunda; higher with lower doses of the extract with a peak at the lowest dose of 100mg/kg body weight. There was a dose dependent increase in GSH. The increases in the concentration of antioxidant biomarkers was statistically significant at $p \leq 0.05$ level compared to the control except at the dose of 600mg/kg body weight in GSH estimations. The results obtained in this study suggest that stem bark extract of *A. floribunda* has a positive effect on antioxidant status at low to moderate doses and causes less increase in lipid peroxidation.

Keywords: *Allabnlackia floribunda*, Glutathione reductase, lipid peroxidation, superoxide dismutase, malondialdehyde.

Article History: Received: March 21, 2020; Revised: April 11, 2020; Accepted: April 30, 2020.

Corresponding Author: Dr. Gabriel-Brisibe C.U *

Department of Medical Biochemistry, College of Medical Sciences,

Rivers State University, Nigeria. Email: Christine.gabrielbrisibe@ust.edu.ng

1. INTRODUCTION

Allanblackia *floribunda* (Oliver or tallow-tree), is one out of nine identified species of the genus Allanblackia and it is a fruit tree of Clusiaceae family or Guttiferae [1]. It is found in the rain forest of South and Equatorial rain forest in Nigeria, to the Central African Republic and East of the Democratic Republic of Congo, and South to Northern Angola [2]. The species of Allanblackia found in certain African countries include *Allanblackia Floribunda*, found in Nigeria, the *Allanblackia Stuhlmanii* found in Tanzania and *Allanblackia Parviflora* found in Ghana. *A. floribunda* has been in use for its medicinal value and noted to prevent the increase of malondialdehyde (MDA) and the decrease of reduced glutathione (GSH) concentration in aorta, heart, kidney and liver of alcohol-induced hypertensive rats and sucrose-induced hypertensive rats [3]. Malondialdehyde is a common marker of oxidative stress and the antioxidant status of humans [4]. It is the most generally used agent to determine the extent of lipid peroxidation [5]. Superoxide dismutase and glutathione and catalase make the first line of defense against harmful radicals released in the body and are the most powerful antioxidants in the body. [6]. Superoxide dismutase and glutathione are biological markers of oxidative stress in the body [7] (Edwin et al, 2013) Allanblackia species have been shown to have medicinal effects. Some Allanblackia species extracts of leaves, roots, fruits and flowers have shown antibactericidal activities. *Allanblackia gabonensis* for example has been reported to display broad spectrum activities of about 72 % against some bacterial strains and fungi [8] [9]. Some *Allanblackia* species investigated over the years have showed significant anti-inflammatory activities as exemplified by the invivo anti-inflammatory study conducted by Nguemfo et al [10]. *Allanblackia floribunda* has demonstrated some antioxidant properties. Kuete et al. isolated some compounds from the bark of *Allanblackia floribunda* which include Allanxanthone, benophenones, 1,7- dihydroxyxanthone, xanthones, morelloflavone bioflavonoids and O-glucoside. They are said to exhibit a wide range of pharmacological activities including antioxidant, anti-inflammatory, anticancer and antimicrobial effects [11] There is a general belief amongst consumers around the world that medicinal herbs are inherently safe, because they are “natural”. However, just because the product is natural does not assure their safety [12][13][14][15]. Any pharmaceutically active agent has the potential to result in synergistic or antagonistic interaction when consumed with other pharmaceutically active compounds. Increased self-medication also increases the chance of adverse reactions to these products as well as adverse drug/herb interactions [12]. This study therefore, investigates the effect of *Allanblackia floribunda* methanol stem bark extract on lipid peroxidation and some antioxidant biomarkers (super oxide dismutase and reduced glutathione) in male albino rats. The significance of this study is to provide scientific evidence of the subacute effects of *Allanblackia floribunda* in male albino rats at graded doses. This is to add to knowledge on the antioxidant effects of *A. floribunda* in its use as a medicinal plant being that there is paucity of information on this.

2. MATERIALS AND METHODS

The following apparatus were used for the experiment: Plain and Universal Containers, EDTA containers, syringes, pH meter, test tubes (pyrex, england), test tube racks, spectrophotometer (spectrumlab), cuvette, centrifuge (harris england), beakers, water bath (hh-4), gavage, cotton wool, foil paper, spatula, funnel, weighing balance (s. mettler). Stopwatch, glass rod stirrer, magnetic stirrer, pestle and mortar, measuring cylinder, micropipettes. The reagents used for this research were of analytical grade. They include: reduced glutathione (GSH), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,4,6-tripyridylstriazine (TPTZ), Thiobarbituric acid (TBA), 5,5-dithiobis-2-nitrobenzoic acid (DTNB, hydrochloride were bought from Sigma Aldrich (Merck), 0.9% Normal saline, Chloroform (May and Baker, Dagenham, England) and Formalin.

The plant stem bark was collected based on local claims by traditional healers of their efficacy in the treatment of malaria in Southern Nigeria. Parts of *Allanblackia floribunda* plant including the stem were collected during the rainy season, from a forest area at Okhumwun village, Benin City, Nigeria. The plant was authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. Voucher specimen of the sample (UBHA$_{361}$) was deposited at the herbarium of the same department. Stem barks of *A. floribunda* plant were obtained fresh, peeled off washed and air dried in the laboratory. The dried stem barks were macerated and 500g of powder was soaked in 2.5 liters of methanol in air tight containers for 3 days and it was stirred occasionally. After which they were filtered using Whatman No 1 filter paper into a clean flask, the filtrates were concentrated using a rotary evaporator (RE 300, Bibby Scientific, UK) and final concentrate was obtained using silica gel. The extracts were thereafter stored in a sterile container and kept at 4^{o}C till when needed. Twenty eight Healthy male albino rats weighing between 115-140 g were obtained from Department of Anatomy, University of Benin, Benin City and were used for the experiments. The rats were housed under standard laboratory conditions at temperature 27±2^{o}C, relative humidity 70 % and at 12 hr day/night cycles in the Department of Biochemistry, University of Benin. They had free access to grower mash and water. The animals were allowed to acclimatize for one week before commencing the experiment. The experiments were conducted in strict compliance with internationally accepted principles for laboratory animals' use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (Ernest *et al*., 1993).

The experimental design for the study was as follows:

- Twenty eight rats were divided into 4 groups after the acclimatization period. The rats were randomly selected on the basis of their weight and each group had 7 rats. Treatment was done once daily for 14 days.
- Group 1 served as the control and was given 1ml Carboxyl Methyl Cellulose (0.7 % CMC) orally.

- Group 2 was administered 100 mg/kg b. wt. of *A. floribunda* stem bark extract dissolved in 1ml of 0.7 % CMC.
- Group 3 was administered 300 mg/kg b. wt. of *A. floribunda* stem bark extract dissolved in 1ml of 0.7 % CMC.
- Group 4 was administered 600 mg/kg b. wt. of *A. floribunda* stem bark extract dissolved in 1ml of 0.7 % CMC.
- Animals were fasted overnight and sacrificed by cervical dissection on day 15 of the experiment. Samples were then collected.

Next there was preparation of Tissue Homogenates. Liver tissues were harvested after sacrificing the animals, weighed and placed in plain containers which contained 10 ml of phosphate buffered saline and stored in ice (4 ^{o}C). 1 g of liver tissue was homogenized in 5 ml of ice cold physiological saline to obtain homogenate. The resulting homogenates were centrifuged at 4500 rpm for 15 minutes and the supernatant obtained used for subsequent analysis. The Estimation of Malondialdehyde (MDA) was carried out using the Buege and Aust (1978) method [16].The principle behind this method is that the MDA formed as an end product of lipid peroxidation reacts with thiobarbituric acid in Trichloroacetic acid (TCA)-2-thiobarbituric acid (TBA)–HCl reagent producing thiobarbituric acid reactive substance (TBEARS), a pink chromatogen which can be measured spectrophotometrically at 532nm.

The MDA concentration of sample was calculated using the extinction coefficient of 1.56 x 10^5m-1cm-

So that mathematically,

$$MDA = \frac{AbxVx1000}{Axvx1xY}$$

Where Ab = Absorbance of sample test at 535 nm

V= Total volume of the reaction mixture =3 ml

A= Molar estimation co-efficient of product = 1.56 x 10^5 m $^{-1}$cm $^{-1}$

l = Light path = 1 cm

v = Volume of sample used=1 ml

Y= Weight of tissue in the volume of sample used (g)

The Determination of Superoxide Dismutase (SOD) Activity was by the method of Misra and Fridovich (1972) [17].The principle used in this method is the ability of SOD to inhibit the auto-oxidation of epinephrine at pH 10.2 making this reaction a basis for a simple assay for superoxide dismutase. Superoxide radical causes the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per superoxide radical introduced increases with increasing pH and concentration of epinephrine.

Calculation after Spectrophotometry

% inhibition = 100 – (100 × Increase in absorbance per min for sample)

Increase in absorbance per minute for blank

1 unit of SOD activity was given as the amount of SOD necessary to cause 50 % inhibition of the auto-oxidation of epinephrine.

Y = mg of protein in the volume of sample used

Estimation of Reduced Glutathione (GSH) was done using the method of Beutler *et al.* (1963) [18]. The principle behind this method is that the reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable (yellow) colour when 5',5'– dithiois – (2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione, 2 – nitro-5-thiobenzoic acid possesses a molar absorption at 412nm. Statistical analyses of the results were carried out. The various results obtained from this study were expressed as Mean ± SEM. One way analysis of variance (ANOVA) followed by LSD and Duncan test was used to determine significance of the differences between the groups. Statistical significance was declared when P value was less than 0.05. The statistical analysis was performed using the statistical package for social science (SPSS) for windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

The effect of *A. floribunda* stem bark extract after oral administration is shown in the bar charts below.

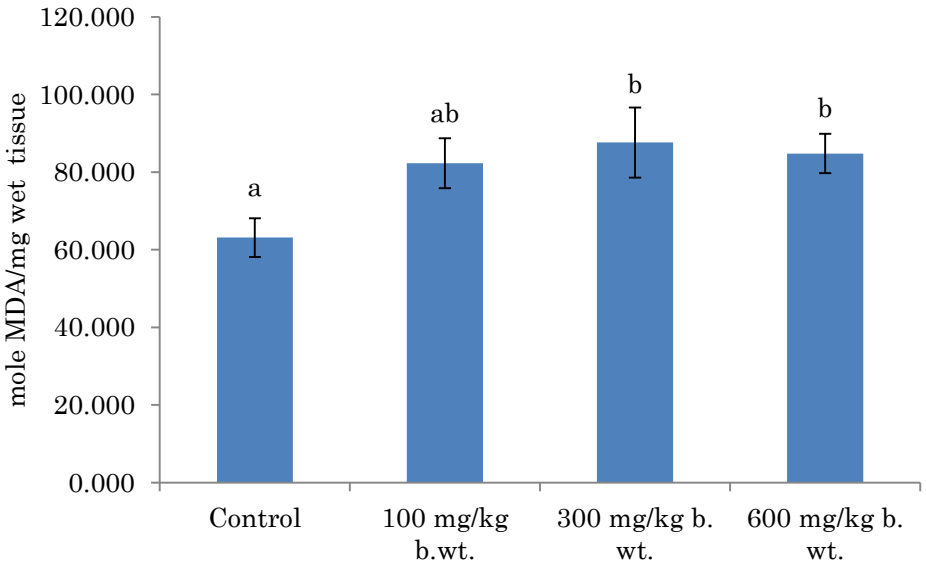

Fig 1: Hepatic lipid peroxidation measured as malondialdehyde (MDA) generated in liver homogenates.

Data are presented as means± SEM (n = 7). Bars with same letters are not significantly different while those with different letters are significantly different at p≤0.05 level

There was a dose dependent increase in MDA concentration. The grouped administered 300 and 600 mg/kg body weight of plant extract had a statistically significant increase in MDA level compared to the control.

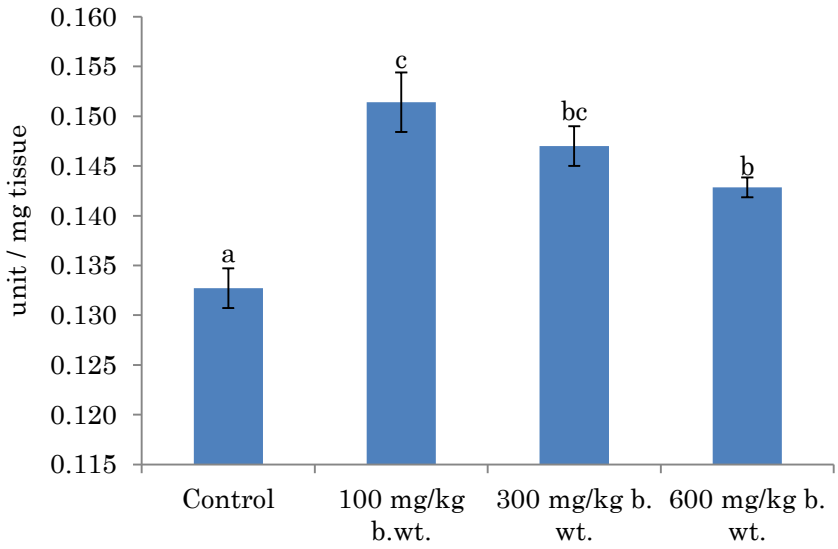

Fig 2: SOD activity in liver homogenate

Data are presented as means± SEM (n = 7).

Bars with same letters are not significantly different while those with different letters are significantly different at p≤0.05 level

A gradual fall in SOD activity was noticed as the extract concentrations steadily increases. The group administered 100 mg/kg body weight had the highest SOD activity (0.15 unit/mg tissue).

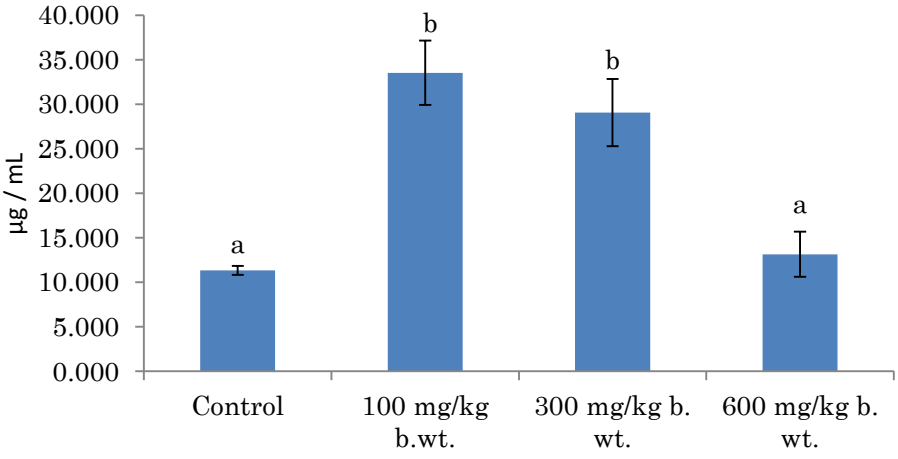

Fig 3: Effect of *Allanblackia floribunda* on estimation of reduced Glutathione (GSH) activity of liver homogenate

The results are express as mean ± SEM for 7 rats per group. Bars with different letters are significantly different while those with same letters are not significantly different at p≤.0.05 level

There was a dose dependent increase in GSH which showed a statistically significant difference at

p≤0.05 level from the group administered plant extracts. 100 and 300 mg/kg extract concentrations caused an upsurge in GSH (33.54 mg/ml and 29.065 mg/ml) when compared to control (11.35 mg/ml) and the group that had highest dose of extract (13.15 mg/ml) having the least increase in GSH concentration.

DISCUSSION

The hepatic content of MDA is used as an indicator of liver tissue damage involving a series of chain reactions [19]. There was an increase in hepatic MDA of the group administered plant extract relative to control (fig1). An increased MDA level in liver suggests enhanced lipid peroxidation induced by *A. floribunda* which is not in keeping with the study by Bilanda et al where MDA levels were reduced by *A.floribunda extract* [3] Superoxide dismutase is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O^{2-}) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Hydrogen peroxide is a harmful by-product of many normal metabolic processes. For prevention of damage, it must be rapidly converted into other less toxic substances. SOD is the primary catalytic cellular defence that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives. SOD can be induced when cells are exposed to agents that stimulate oxidative stress. The primary function of SOD is to scavenge O_2 radicals (to molecular O_2 and H_2O_2) generated in various physiological processes, thus preventing the oxidation of biological molecules, either by the radicals themselves, or by their derivatives [20]. The result shows a statistically significant increase in SOD activity of the groups given extract when compared to the control at p≤0.05 level. The group administered 100 mg/kg b. wt. had the highest SOD activity and least MDA level. Hence, the SOD may have been produced to combat the free radicals generated. This result further suggests that the extract may be safer at lower doses. GSH is a tripeptide (L-γ-glutamyl cysteinyl glycine), antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification from reactive oxygen species, conjugation and excretion of toxic molecules and control of the inflammatory cytokine cascade [21]. GSH is used as a cofactor by multiple peroxidase enzymes to detoxify peroxides generated from oxygen radical metabolism [22]. In the present study, alteration in hepatic GSH has a similar trend to SOD activity i.e., gradual decrease in *A. floribunda* concentration significantly caused a steady increase in cellular (hepatic) GSH levels. Further suggesting that A. floribunda has better antioxidant effects at lower doses and less lipid peroxidation.

4. CONCLUSION

The results obtained in this study suggest that the stem bark extract of *A. floribunda* has a positive effect on antioxidant status at low to moderate doses. At 100 to 300 mg/kg body weight there was less increase in lipid peroxidation, increase in SOD concentration and upsurge in hepatic GSH tripeptide. However, Further studies should be conducted to ascertain the effect of prolonged use of high concentrations of this plant as the possibility of chronic toxicity has not been ruled out.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals were abused during the study

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

None.

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

Authors have no conflict of interest.

REFERENCES

1. Fobane JL., Ndam EN, Mbolo M. - Population structure and natural regeneration of *Allanblackia floribunda* oliv. (Clusiaceae) in a forest concession of East Cameroon. Journa. Biodivers. Environ.Sci. 2014; 4(2):403- 410.
2. https://uses.plantnet-project.org/en/Allanblackia_floribunda_(PROTA) Accessed on 17th April, 2020.
3. Bilanda DC, Dimoa T, Djomenia P D D, Bella NMT., Aboubakar OBR, Nguelefack TB., Tana, PV, and Kamtchouinga, P- Antihypertensive and antioxidant effects of *Allanblackia floribunda* Oliv. (Clusiaceae) aqueous extract in alcohol- and sucrose-induced hypertensive rats. Journal Ethnopharmacol.2010; 128:634-640.
4. Gawel S, Wardas M, Niedworok E, Wardas P- Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek. 2004; 57(9-10): 453-455.
5. Deepa D'souza, Babu G Subhas, Preethi Balan -Estimation of serum malondialdehyde in potentially malignant disorders and post-antioxidant treated patients: A biochemical study.ContempnClin Dent. 2012; 3(4):448-451.
6. Ighodaro OM, Akinloye OA- First line defence anti-oxidants- superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid . Alexandria Journal of Medicine. 2018; 54: 287-293
7. Edwin Ho, Keyvan Karimi Galougahi, Chia-Chi Liu, Ravi Bhindi, Gemma A Figtree- Biological Markers of Oxiative Stress: Applications to Cardiovascular Research and Practice; Redox Biol. 2013; 1(1): 483-91.
8. Fankam A, Kuiate J R, Kuete V- Antibacterial and antibiotic resistance modifying activity of the

extracts from *Allanblackia gabonensis*, Combretum molle and Gladiolus quartinianus against Gram-negative bacteria including multi-drug resistant phenotypes. BMC complementary and alternative medicine.2015; 15:206.

9. Ajibesin KK, Rene N, Bala DN, Essiett UA- Antimicrobial activities of the extracts and fractions of *Allanblackia floribunda*. Biotechnology.2008; 7:129-133.
10. Nguemfo E L, Dimo T, Azebaze AGB. *et al*.. Anti-inflammatory and anti-nociceptive activities of the stem bark extracts from *Allanblackia monticola* STANER LC (Guttiferae). J. Ethnopharmacol. 2007; 114:417-24.
11. Kuete V, Azebaze A, Mbaveng A, Nguemfo E, Tshikalange E, Chalard P, Nkengfack A- Antioxidant, antitumor and antimicrobial activities of the crude extract and compounds of the root bark of *Allanblackia floribunda*. Pharmaceutical Biology. 2013; 49:57-65.
12. Matthews BH., Lucier WG. and Fisher DK- Medical herbs in the United States: Research needs. Environmental Health Perspectives.1999; 107(10):773-778.
13. Colombo ML., Assisi F, Puppa TD, Moro P, Sesana FM, Bissoli M, Borghini, R, Perego S, Galasso G, Banfi E, Davanzo F- Most commonly plant exposures and intoxications from outdoor toxic plants. Journal of pharmaceutical sciences and research. 2010; 2 (7):417-425.
14. Philomena G- Concerns regarding the safety and toxicity of medical plants – an overview. Journal of applied pharmaceutical science. 2011; 01(6):40-44.
15. Staines SS- . Herbal medicines: adverse effects and drug-herb interactions. Journal of the Malta college of pharmacy practice. 2011; 17:38–42.
16. Beuge JA and Aust SD- Microsomal lipid peroxidation. Methods in enzymology, 1978; 53: 302-310.
17. Hara p. Misra, Irwin Fridovich-The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of Biological Chemistry. 1972; 247(10): 3170-3173.
18. Beutler E, Duron O, Kelly BM-Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–890
19. Abdullah N, Saat, NZM., Hasan HA., Budin SB, Kamaralzaman S- Protective effect of the ethanol extracts of *Zingiber officinale* on paracetamol induced hepatotoxicity in rats. Malaysian Journal of Health Science. 2004; 2(2):85-95.
20. Zelko I, Mariani T, Folz R- Superoxide dismutase multigene family: A comparisom of the CuZn-SOD (SOD 1), Mn-SOD (SOD 2), and EC-SOD (SOD 3) gene structures, evolution, and expression._Free Radical Biology_Med. 2002;33:337-49.
21. Brown, LA- Chronic ethanol ingestion and the risk of acute lung injury. Alcohol. 2004; 33: 191 – 197.
22. Strange RC, Jones PW, Fryer AA. Glutathione Stransferase: genetics and role in toxicology. Toxicology Letters. 2000; 112-113: 357–363.