

The protective of combined extracts of *Gongronema latifolium* and *Nauclea latifolia* on hepatotoxicity in rats.

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ABSTRACT:

Aims:

Protective effects of the combined ethanolic leaf extracts of *Gongronema latifolium* (GL) and *Nauclea latifolia* (NL) on acetaminophen-induced liver toxicity were studied in rats in comparison with individual extract treatment and the standard drug; Silymarin.

Study design: The design consisted of sixty rats divided into ten groups of six rats each. Hepatotoxicity was induced orally with a single dose of acetaminophen (200mg/kg, bw, p.o.) diluted with sucrose solution (40% w/v) in five groups. Animals were then separated into five hepatotoxic and five non-hepatotoxic groups and treated with GL, NL, GL+NL, Silymarin or distilled water.

Place and Duration of Study: Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria, between February, 2010 to August, 2011.

Methodology: The phytochemical constituents of *Nauclea latifolia* (NL) and *Gongronema latifolium* (GL) leaf extracts were determined quantitatively using standard methods. The pharmacological studies involves ten groups of six rats each; divided into five hepatotoxic and five non-hepatotoxic groups and then treated with the extracts, silymarin or distilled water. Biochemical indices (protein, albumin, AST, ALT) as well as antioxidant enzymes like catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and histological examinations were done with the serum and liver respectively.

Results: The quantitative phytochemical investigations of the NL and GL leaf extract showed the content of alkaloids, polyphenols, flavonoids, saponins and hydrocyanic acid. Administration of only toxicant showed that the ALT and AST levels were significantly ($P=0.05$) increased by 3.19 and 3.01 fold respectively while the levels of total protein and albumin were decreased when compared to the normal control. Pretreatment with extracts both singly and in combination for 21 days decreased the elevated levels of the transaminases and restored the normalcy of total protein and albumin significantly. These were most impacted in the combined extract treatment group, indicating a positive synergy. In only acetaminophen treated rats, CAT (441.47 ± 130.95), GPx (2720.27 ± 33.78) and SOD (13.52 ± 0.21) were decreased significantly but treatment with extracts of GL (CAT = 531.37 ± 27.60 , GPx = 2768.51 ± 19.78 & SOD = 15.05 ± 0.35) or NL (CAT = 475.20 ± 50.13 , GPx = 3307.26 ± 343.69 & SOD = 13.69 ± 0.41) and in combination (CAT = 785.40 ± 74.46 , GPx = 4449.59 ± 26.48 & SOD = 15.69 ± 0.60) caused a significant increase ($P=0.05$) in these antioxidants activities. Acetaminophen treatment alone showed severe liver damage while pretreatment with only NL or GL showed an improvement in histological section of the liver but the protective effect was more pronounced in the combined extract treatment group against the hepatocellular damage.

Conclusion: These results suggested that NL and GL leaf extracts have a significant role in alleviating liver damage, with the combined extracts synergistically improving the levels of these indices more, thus suggesting a better amelioration of hepatotoxicity.

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Key words: Hepatotoxicity, *Nauclea latifolia*, *Gongronema latifolium*, combined extracts, Acetaminophen, Silymarin, antioxidants.

1. INTRODUCTION:

Herbal therapy is a fundamental aspect of traditional medicine that primarily uses medicinal plants preparations to prevent and improve health and for the management of ailments [1]. Most of our traditional societies in Africa and elsewhere have always used herbs and other plant parts to promote healing of various diseases [2]. In the recent past, people who are sick consulted physicians for treatment, however this is gradually changing now. According to WHO, 2003 [3], more and more people around the world are turning to herbal medicine because of the following reasons: Herbal medicines are natural, potent and have minimum aftermath effect; herbal medicines are relatively cheap and affordable to many people; the medicinal plants are readily available around us and treatments with herbal medicines in most cases are long lasting or permanent. However a number of these plants are yet to be scientifically evaluated for their efficacy and safety. The consensus guidelines for research on complementary and alternative systems have recommended that traditional

systems of medicine be in their intact form as clinically used (rather than as isolated components) for clinical efficacy and overall safety [4].

Gongronema latifolium (*Asclepiadaceae*) is a perennial edible plant with soft and pliable stem. It is widely used in the West African sub-region for a number of medicinal and nutritional purposes [5]. In Eastern states of Nigeria, the plant locally known as 'utazi' is a popular spice. The leaves are used to prepare food for mothers that have recently put to bed where it is believed to stimulate appetite, reduce post-partum contraction and enhance the return of the menstrual cycle [6]. On the other hand, *Nauclea latifolia* is from the family *Rubiaceae*. The plant is commonly known as Pincushion tree. *Nauclea latifolia* is used profusely by traditional medicine practitioners. The bark and root of *N. latifolia* are used for the treatment of malaria in Ghana [7]. The leaf is also used for the treatment of malaria in East Africa [8] and in Nigeria [9]. The crude extract of the roots have been shown to have anti-hypertensive effect [10].

Human beings are exposed on a daily basis to certain toxic chemicals and pathogens, which cause certain serious health problems. Certain chemicals and reagents that were thought to be health friendly have been proved to have serious adverse effects on health. Amongst these toxic substances is acetaminophen. Acetaminophen, a most commonly used analgesics, effectively reduces fever and mild-to moderate pain, and is considered to be safe at therapeutic doses. However, acetaminophen overdose causes severe hepatotoxicity that leads to liver failure in both humans and experimental animals [11, 12].

Silymarin has been used for over 20 years in clinical practice for the treatment of toxic liver diseases [13]. *Silymarin* extract from the seeds of the plant *Silybum marianum*, also called "milk thistle", has been described to be an antioxidant and exhibits anticarcinogenic, antiinflammatory, hepatoprotection and growth modulatory effects [14, 15]. In this study, *silymarin* was used as a positive control against the acetaminophen-induced hepatic damage in rats.

The combination of herbs or photochemical from more than one source has proven to be more useful and beneficial in management of various ailments including those that seem to defile conventional medication [16], as synergistic effects among individual components are considered essential to the herbal remedy approach to treatment and prevention of diseases. Thus this work focused on the usage of combined herbal therapy in ameliorating the adverse effect of acetaminophen to the liver, having visualized the prominent functions of the liver for survival. There is no evidence tendered as regards the remedial effects of the leaves of NL on liver malfunction, but GL has been reported by Johnkenedy and Emejulu, 2011[17] on its protective role on hepatic toxicity in Wistar rats. In view of the attributes stated for the two plants above and their antioxidant status [18, 19], this work reposes on the *in vivo* determination of the effects of combined ethanolic extract of the leafs of *Nauclea latifolia* and *Gongronema latifolium* on hepatocellular damage.

2. MATERIALS AND METHODS

2.1. Chemicals

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were obtained from Cayman Chemical Company, U.S.A. The routine assay Kits for total protein, albumin and the aminotransferases, were purchased from Randox Laboratories Ltd. United Kingdom, *Silymarin* was purchased from Vellore, India, while acetaminophen (APAP) was obtained from Sigma Chemical (St. Louis, MO, USA).

2.2. Plant materials and extraction

Fresh but matured *Gongronema latifolium* leaves were collected from a cultivated land at Ibiaku Itam, Nigeria while *Nauclea latifolia* was collected in Calabar, Nigeria in March, 2012. They were authenticated by Dr. E. G. Amanke in the Department of Botany, University of Calabar, Nigeria and voucher specimen deposited in the Department of Botany herbarium, University of Calabar. The wet method of extraction according to Effiong et al., 2012 [20] was adopted for the preparation of the ethanol extract.

2.3. Phytochemical evaluation

NL and GL leaves were subjected to quantitative analysis for various phytoconstituent like Cyanogenic glycosides determined by the alkaline picrate colorimetric method of Harbone, (1998) [21], saponins in which the method of Obadoni and Ochukko, 2001 [22] as described by Okwu, 2004 [23] was employed, phenols was done according to the Folic- ciocaltean colorimetric method of A.O.A.C, 1990 [24], tannins was determined by colorimetric method of Trease and Evans,1996 [25], flavonoid was done using the ethyl acetate gravimetric method of Reitman and Frankel, 1957 [26] while alkaloid determination was done using the alkaloid precipitation gravimetric method described by Harbone, (1998) and Okwu, 2004 [21, 23].

2.4. Experimental animals

Male Albino Wistar rats of (120-150g) obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy of the University of Uyo, Nigeria were used. They were kept in clean cages (wooden bottom and wire mesh top), maintained under standard laboratory conditions (Temperature 25± 5oC, Relative humidity 50-60%, and a 12/12h light/dark cycle) and were allowed free access to standard diet (Vital Feed from Grand Cereals & Oil Mills Limited, Jos, Plateau State of Nigeria) and water *ad libitum*. Animals were acclimatized for 14

days in the animal house of the Department of Biochemistry, University of Calabar, Nigeria and then divided into groups for experimentation.

2.5. Experimental design

The design consisted of sixty rats divided into ten groups of six rats each. Hepatotoxicity was induced in five groups orally with a single dose of acetaminophen (200mg/kg, bw, p.o,) diluted with sucrose solution (40% w/v). Animals were then separated into five hepatotoxic and five non-hepatotoxic groups and treated as shown in Table 2. Treatment was done twice daily (6.00a.m and 6.00p.m) except in the group treated with Silymarin that was done once (6.00a.m). At the end of 21 days, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles, emptied into plain tubes and allowed to clot for about two hours. The clotted blood was thereafter centrifuged at 3,000rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile syringes and needles and stored frozen until used. Prior to dissection, the liver tissue was perfused in heparinized saline (0.9% NaCl) to remove any red blood cells and clots and thereafter blotted with blotting paper. The tissue was then suspended in boun’s fluid for histologic evaluation.

Table 1: Experimental design.

Hepatotoxic rats			Non-Hepatotoxic rats		
Group	No. of Animals	Treatment	Group	No. of Animals	Treatment
1	6	Acetaminophen (2g/kg bw,p.o.)+ GL (400mg/kg b.w) =GL _H	1	6	GL extract (400mg/kg b.w) =GL _{NH}
2	6	Acetaminophen(2 g/kg, bw, p.o.) + NL (400mgkg/b.w) = NL _H	2	6	NL extract (400mgkg-1b.w) = NL _{NH}
3	6	Acetaminophen(2 g/kg, bw, p.o.) + GL+NL (200mgkg/b.w) = (GL+NL) _H	3	6	GL+ NL extract (400mgkg-1b.w) = (GL+NL) _{NH}
4	6	Acetaminophen(2g/kg,bw,p.o.) +Silymarin commercial drug (100mg/kg, bw, p.o.) =SILYMARIN _H	4	6	Silymarin commercial drug (100mg/kg, bw, p.o.) =SILYMARIN _{NH}
5	6	Acetaminophen (2 g/kg, bw, p.o.) diluted with sucrose solution (40%w/v). (Hepatotoxic control) =HC	5	6	Placebo (Normal control) = NC

H =Hepatotoxic rats, NH = Non- Hepatotoxic rats

2.6. Biochemical analysis

Serum was used for the estimation of various biochemical parameters: ALT and AST were estimated using the method of [Reitman and Frankel, 1957](#) [26], albumin, with the method of [Mallay and Evelyn, 1937](#) [27], total protein using [Lowry, 1951](#)'s method [28] and antioxidants analysis using Spectrophotometer assay kit from Cayman Chemical Company; for superoxide dismutase (SOD) based on the method of [Nebot et al., 1993](#) [29], catalase (CAT) involves the method of [Aebi, 1984](#) [30] and glutathione peroxidase (GPx) was estimated using [Paglia and Valentine, 1967](#)'s method [31].

2.7. Histopathological examination

Liver tissues were preserved in 10% formaldehyde solution. The tissues processed and embedded in paraffin wax. Sections of about 4-6 microns were made from the fixed liver and stained with basic dyes, of Heamatoxylin and Eosin (H&E) according to Conn,(1946) [32] procedure and photomicrographs (x 400) developed.

2.8. Statistical analysis

The results were analyzed by one-way ANOVA, using SPSS statistical package. All data were expressed as Mean $\pm$ SE and difference between groups considered significant at $P=0.05$.

3. RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1. Preliminary phytochemical investigation

The phytochemical composition of the leaves of NL and GL are shown on Table 2. NL showed a higher content of flavonoids (1.52 ± 0.02), saponins (17.52 ± 0.02), polyphenols (2.00 ± 0.01) and alkaloid (7.07 ± 0.02) while tannins were higher in GL (2.04 ± 0.02). Hydrocyanic acid was comparable in the two plants (GL= 13.29 ± 0.02 , NL= 13.20 ± 0.02). However there appear, to be a complement of biochemicals in the leaves of these plants.

3.1.2. Serum biochemical parameters

Serum aminotransferase activities (AST and ALT) were raised significantly ($P=0.05$) by 3.19 and 3.01 fold respectively in the hepatotoxic control (HC) relative to normal control (NC). Twenty one days treatment caused a significant reduction in AST and ALT of GL_H (41.33 ± 2.01 & 18.27 ± 5.01), NL_H (50.33 ± 12.23 & 14.33 ± 1.38), (GL+NL)_H (32.67 ± 1.17 & 10.00 ± 0.73) and Silymarin_H (ALT= 46.67 ± 1.72) in relative to HC (70.67 ± 0.42 & 69.33 ± 18.09) [Table 3]. Result of total protein levels and albumin concentration in serum of GL_H (5.95 ± 0.61 & 22.86 ± 0.03), NL_H (7.74 ± 0.13 & 28.71 ± 0.08), (GL+NL)_H (8.65 ± 0.08 & 42.10 ± 0.11), Silymarin_H (6.73 ± 0.06 & 42.45 ± 0.11) and HC (5.98 ± 0.34 & 2.69 ± 0.11) recorded a significant decrease ($P=0.05$) in comparison with NC (9.24 ± 0.92 & 66.05 ± 8.38). Also this result indicated a significant decrease in albumin of GL_H (22.86 ± 0.03) and NL_H (28.71 ± 0.08) in relative to (GL+NL)_H (42.10 ± 0.11), but NL_H (7.74 ± 0.13) and (GL+NL)_H (8.65 ± 0.08) indicated a significant increase in total protein relative to Silymarin_H (6.73 ± 0.06) [Table 3].

3.1.3. Hepatic oxidative stress parameters

Antioxidants analysis showed HC produced a significant decrease in GPx (2720.27 ± 33.78) and SOD (13.52 ± 0.21) activities while CAT (441.47 ± 130.95) showed a significant increase when compared to NC (GPx= 7992.09 ± 338.28 , SOD= 52.56 ± 3.96 while CAT= 329.67 ± 5.18) implying hepatotoxicity imposed oxidative stress. GPx, SOD and CAT activities were significantly increased in GL_H (2768.51 ± 19.78 , 15.05 ± 0.35 & 531.37 ± 27.64), NL_H (3307.26 ± 343.69 , 13.69 ± 0.41 & 475.20 ± 50.13) and (GL+NL)_H (4449.59 ± 26.48 , 15.69 ± 0.60 & 785.40 ± 74.46) in comparison to HC (2720.27 ± 33.78 , 13.52 ± 0.21 & 441.47 ± 130.95). The effect of Silymarin_{NH} was similar with GL_{NH} and NL_{NH} in GPx activity but (GL+NL)_{NH} (7408.46 ± 7.66) indicated a significant increase ($P=0.05$) compared to SILYMARIN_H (2648.67 ± 12.47). CAT activity of GL_{NH} (7376.37 ± 38.80) was significantly decreased when compared with both SILYMARIN_{NH} (8016.76 ± 97.14) and (GL+NL)_{NH} (7408.46 ± 7.66). NL_{NH} (322.83 ± 7.70) also caused a significant decrease in CAT activity compared to (GL+NL)_H (785.40 ± 74.46) [Table 4].

3.1.4. Histopathological examination.

The cellular architecture and integrity of the hepatocytes were also examined in this study.

Hepatic control liver (HCL) showed severe hepatic fatty change, sinusoidal dilation and congestion, mild periportal inflammation, fibrosis, severe feathery degeneration and necrosis in comparison to normal control liver (NCL). NL_H liver (NL_HL) showed an improvement in histological section, GL_H liver (GL_H L) improves over NL_H liver (NL_H L) and HCL while (GL+NL)_H liver (GL+NL)_H L showed hepatocytes similar to the NC rat's liver histology and compared well with SILYMARIN_H liver (SILYMARIN_HL) (FIG.1-5 (a & b)).

3.2. Discussion

The knowledge of phytochemical composition is generally fundamental to the understanding of the modes of action of medicinal plants in addition to their roles in human and animal nutrition; it is the diverse composition of these components in plants that places them at an advantaged position over and above chemotherapy [33]. The results of quantitative analysis of phytochemical compounds investigated in this study revealed high phenolics and flavonoids in the ethanol leaves extract of *G.latifolium* and *N.latifolia*. Several African medicinal plants have been shown to have hepatoprotective effects [34, 35, 36, and 37]. Hepatoprotection is possibly due to flavonoids which exert a membrane-stabilizing action that protect the liver cells from injury [38, 39]. Some medicinal plants possess hepatoprotective effects. These effects are present because they contain some bioactive compounds [40]. The presence of saponins in a variety of herbal preparations administered to humans proved to be potent against cancer and hepatic cell proliferation [41]. All these facts about the roles of chemical constituents of medicinal plants suggest that *G.latifolium* and *N.latifolia* could exert their protective capacity on hepatocellular damage due to the presence of flavonoids and saponins.

Table 2

Quantitative phytochemical composition of leaves of *G.latifolium* and *N.latifolia*

	Flavonoids (%)	Tannins (%)	Saponins (%)	Polyphenols (%)	Alkaloid (%)	Hydrocyanic acid (HCN) (%)
<i>G.latifolium</i>	0.54±0.02	2.04±0.02	0.66±0.03	0.33±0.00	1.97±0.04	13.29±0.02
<i>N.latifolia</i>	1.52±0.02	0.24±0.00	17.52±0.02	2.00 ± 0.01	7.07±0.02	13.20±0.02

The liver is a major target organ for toxicity of xenobiotics including drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. With respect to acetaminophen dependent hepatotoxicity, it is generally accepted that P450- dependent bioactivation of acetaminophen is a main cause of potentially fulminant hepatic necrosis upon administration or intake of lethal dose of acetaminophen [42, 11].

The increased levels of ALT and AST in *HC* observed in this study was a clear indication of a kind of injury or the other, caused by acetaminophen toxicity ; this is similar to the work of Bartlett, 2004[43] where hepatocellular necrosis or membrane damage leads to very high levels of serum AST and ALT released from liver to circulation. It was in consonance with the work of Taju et al., 2011, John Kennedy and Emejulu, 2011 [44, 17]. There were reduced levels of ALT and AST in all rats treated with the two extracts, however the reduction was better impacted in the combined extract treatment group (*GL+NL*)_H, indicating a positive synergy and was similar to the work of [19], where GL ameliorated the increase in serum AST and ALT levels caused by acute CCl₄ induced hepatotoxicity *in vivo*. Taju et al., 2011 [44] also reported the same effect by GL which is in consonance with the present study.

TABLE 3

Effect of treatment on serum Biochemical indices in non hepatotoxic and hepatotoxic rats.

GROUP	TP(mg/L)	Alb(mg/L)	AST(U/L)	ALT(U/L)
<i>GL</i> _H	5.95±0.61*, b	22.86±0.03*, b, c	41.33±2.01*, a, b	18.27±5.01*, a, b
<i>GL</i> _{NH}	12.36±0.97*	59.93±3.51a,b,c	75.67±15.39*, a,b,c,	35.67±5.68*, a,b,c,
<i>NL</i> _H	7.74±0.13a, b, c	28.71±0.08*, c	50.33±12.23*, a, b	14.33±1.38*, a, b
<i>NL</i> _{NH}	10.31±2.03	44.84±2.30a, c	57.33±8.34*, b, c	49.33±2.59*, a,b,c,
(<i>GL+NL</i>) _H	8.65±0.08*, b	42.10±0.11*, a, b	32.67±1.17*, a, b	10.00±0.73*, a, b
(<i>GL+NL</i>) _{NH}	10.39±1.01	82.94±12.56b	18.00±2.24	68.67±2.47*
<i>SILYMARIN</i> _H	6.73±0.06*	42.45±0.11*	73.33±2.11	46.67±1.72*, a
<i>SILYMARIN</i> _{NH}	12.47±0.47*	57.50±8.99a	12.47±0.47*	57.50±8.99a
<i>HC</i>	5.98±0.34*	2.69±0.11*	70.67±0.42*	69.33±18.09
<i>NC</i>	9.24±0.92	66.05±8.38	19.17±2.52	23.00±4.02

**P*≤.05 vs NC; a = *P*≤.05 vs HC; b = *P*≤.05 vs *SILYMARIN*; c = *P*≤.05 vs *GL+NL* Mean ± SE, n = 6, H = hepatotoxic rats, NH = non- hepatotoxic rats

Total protein and albumin concentrations were significantly decreased (*P*≤.05) in *HC* compared to the NC and was similar with the report by Taju et al., 2011 [44]. Hence a mild impairment in hepatotoxicity could be inferred. *NL*_H and *GL*_H showed significant decrease (*P*≤.05) in total protein and albumin, these were modulated by (*GL+NL*)_H to values comparable to NC and *SILYMARIN*_H. Thus a holistic protection against hepatotoxicity in (*GL+NL*)_H which is not feasible in *NL*_H and *GL*_H has been established in this work.

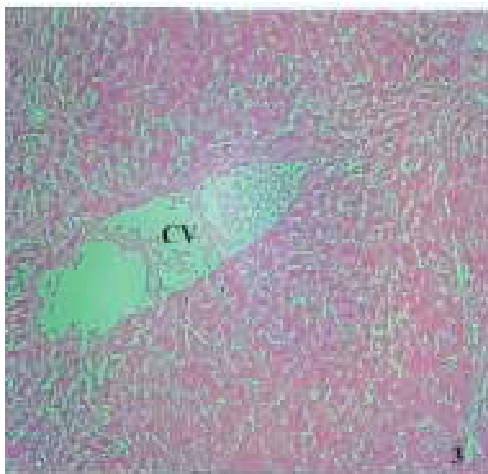
In recent studies acetaminophen was found to induce substantial mitochondrial oxidative stress and peroxy nitrite formation [42]. This oxidative stress preceded cell injury by several hours [45] and free radical scavenger"s attenuated acetaminophen induced liver injury [46]. This was also found in the present study as the antioxidants were significantly reduced in *HC*. *NL*_H and *GL*_H significantly (*P*≤.05) increased the antioxidants activities indicating a possible attenuation of oxidant stress. The combined extracts (*GL+NL*)_H administrations synergistically minimized the production of free radicals and also boost the activities of the scavengers of free radicals, thus minimizing hepatocellular injury produced. This was so because a good liver function is inextricably linked to a good detoxification and antioxidant system, which is indispensable in ensuring optimum physiological function and prevention of diseases. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Thus this could be attributed to the amelioration effects of the GL and NL leaf extracts on hepatocellular damage. The potency of these substances may have been amplified on account of increase concentration or build up of specific phytochemicals present in the combined extracts. The extracts of GL and NL may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds [47], or by increasing the synthesis of antioxidant molecules.

TABLE 4
Effect of treatment on Oxidative Stress in non hepatotoxic and hepatotoxic rats.

	GPx (U/g protein)	SOD (U/g protein)	CAT (U/g protein)
GL _H	2768.51±19.78*	15.05±0.35*	531.37±27.64 b, c
GL _{NH}	7376.37±38.80*, b,	68.40±12.61	308.33±5.47
NL _H	3307.26±343.69*, a, b	13.69±0.41*	475.20±50.13b,c
NL _{NH}	7976.98±73.23c	49.77±13.65	322.83±7.70
(GL+NL) _H	4449.59±26.48*, a, b	15.69±0.60*	785.40±74.46*, a
(GL+NL) _{NH}	7408.46±7.66*, b	50.87±14.20	311.67±17.54
SILYMARIN _H	2648.67±12.47*	16.03±0.45*	884.55±7.85*, b
SILYMARIN _{NH}	8016.76±97.14	54.03±14.64	337.50±8.32
HC	2720.27±33.78*	13.52±0.21*	441.47±130.95*, a
NC	7992.09±338.28	52.56±3.96	329.67±5.18

*P=.05 vs NC; a = P=.05 vs HC; b = P=.05 vs Silymarin; c = P=.05 vs GL+NL Mean ± SE, n = 6, H = hepatotoxic rats, NH = non- hepatotoxic rats

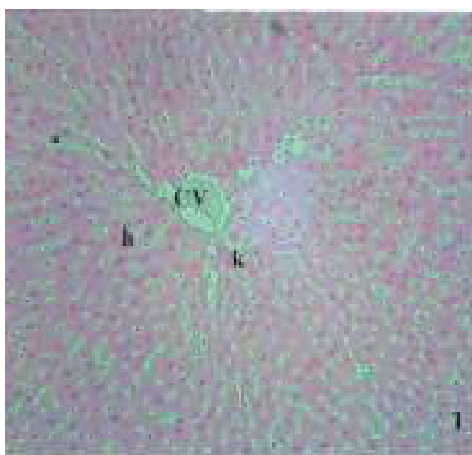
Histologically, result of the HC rats showed that the lobular architecture was maintained, but there was also severe fatty change, sinusoidal dilation and congestion, mild periportal inflammation, fibrosis, severe feathery degeneration and necrosis (FIG. 1(a) HCL). On the other hand, NC liver histology showed normal liver architecture with mild periportal oedema and conspicuous sinusoids with mild steatosis (FIG. 1(b) NCL). These are in consonance with the work of [19, 44]Etim et al., 2008 and Taju, 2001). NL_H showed improvement in histological structure of liver sections, pronounced in a normal appearance of liver lobules with strains of hepatocytes; it compared well with section of HC rat's liver and similar to NL_{NH} (FIG.2 (a) NL_H L, FIG2 (b) NL_{NH}L). The portal areas showed portal veins still distended and engorged with blood. The hepatocytes exhibited some degree of histological regeneration, reduction in fat accumulation, less sinusoids dilatation with decrease in number of Kupffer cells and less necrotic cells were observed. There was an improvement with GL_H and GL_{NH} compared to HC, and NL_H, their lobules were distinctly outlined together with the nuclei, implying an increase in activity of the cells. (FIG. 3 (a) GL_H L, FIG3 (b) GL_{NH}), this was similar with the report of [19](Etim et al., 2008). (GL+NL)_H rats showed hepatocytes similar to NC and (GL+NL)_{NH} histology; liver tissues showing normal architecture and prominent sinusoids and congested blood vessels (FIG. 4 (a) (GL+NL)_H L, FIG4(b)(GL+NL)_{NH} L). These features were similar to those of SILYMARIN_H (FIG. 5 (a) SILYMARIN_H L, FIG5 (b) SILYMARIN_{NH} L) and both compared well with the NC and SILYMARIN_{NH} liver histology.



H&E

FIG. 1 (a) HCL

Section in the liver of hepatotoxic control rat showing loss of the normal architecture with distended central vein (CV)



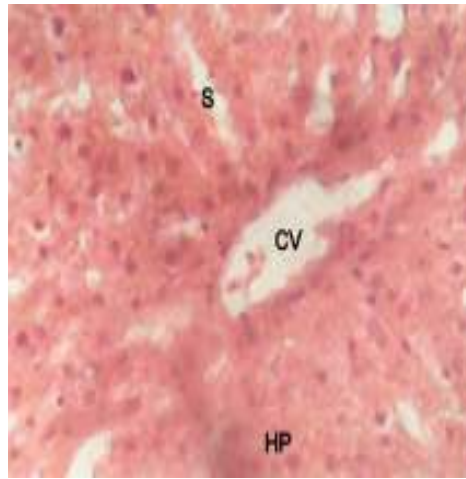
H&E

FIG. 1 (b) NCL

Section in the liver of normal control rat showing hepatic cells (h), Sinusoidal spaces (s) with Kupffer cells (k) and central vein (CV)

FIG. 1 (a&b): Photomicrographs liver of hepatotoxic control and Normal control rats given placebo (X150). H&E= Haematoxiniln and eosin. HCL = Hepatotoxic control liver, NCL = Normal control liver.

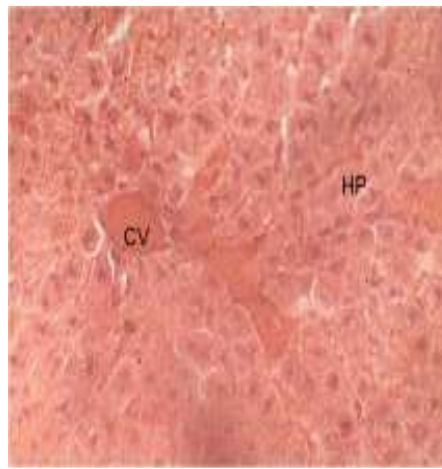
HC = Acetaminophen (2 g/kg, bw, p.o.) diluted with sucrose solution (40%w/v). (Hepatotoxic control)
NC = Placebo (Normal control)



H&E

FIG.2 (a) $NL_H L$

Liver tissue of NL_H rat showing normal architecture with hepatic plates (HP), conspicuous sinusoids(S) and central vein (CV) hyperplasia



H&E

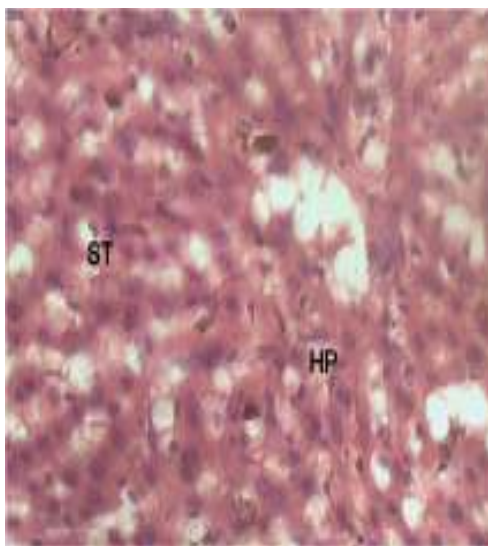
FIG.2 (b) $NL_{NH} L$

Liver tissue showing normal hepatic plate (HP) and central vein (CV).

FIG.2 (a&b): Photomicrographs liver of NL_H and NL_{NH} rats (X400).

H&E=Haematoxylin, $NL_H L$ = NL_H liver, $NL_{NH} L$ = NL_{NH} liver. H=hepatotoxic rat, NH=non-hepatotoxic rat.

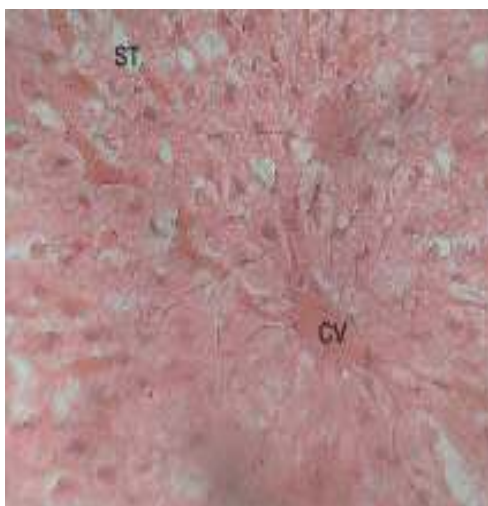
NL_H = Acetaminophen (2 g/kg, bw, p.o.) + NL extract (400mgkg-1b.w), NL_{NH} = NL extract (400mgkg/b.w)
H =Hepatotoxic rats, NH = Non- Hepatotoxic rats



H&E

FIG. 3(a) GL_HL

Liver tissue showing normal hepatic plate (HP) and moderate steatosis (ST).



H&E

FIG.3 (b) GL_{NH}L

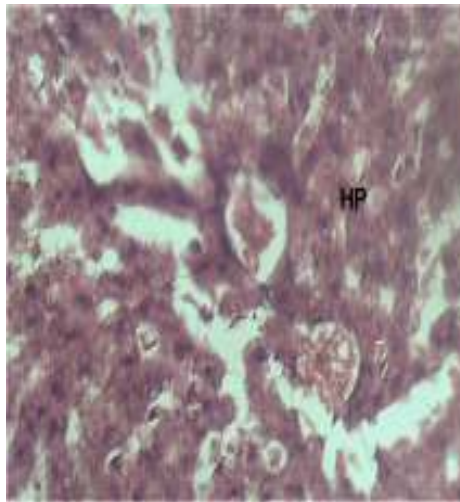
Liver tissue showing central vein (CV) and hepatocytes radiating from the central vein. The hepatocytes show prominent steatosis (ST).

FIG. 3 (a&b): Photomicrographs liver of GL_H and GL_{NH} rats (X400).

H&E=Haematoxylin and Eosin, GL_HL =GL_H liver, GL_{NH}L =GL_{NH} liver. H=hepatotoxic rat, NH=non-hepatotoxic rat.

GL_H =Acetaminophen (2g/kg bw,p.o)+ GL (400mg/kg b.w), GL_{NH} = GL extract (400mg/kg b.w)

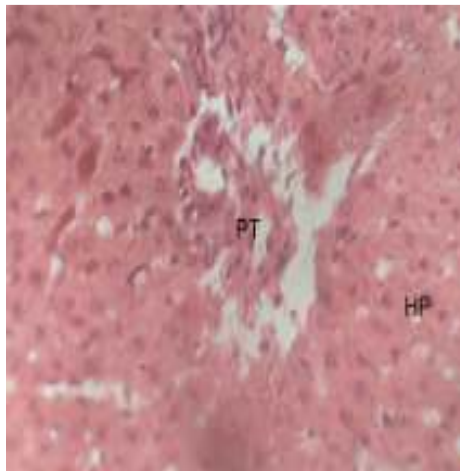
H =Hepatotoxic rats, NH = Non- Hepatotoxic rats



H&E

FIG. 4 (a) (GL+NL)_HL

Liver tissue showing normal architecture with plate of hepatocytes (HP) and congested blood vessels.



H&E

FIG.4 (b) (GL+NL)_{NH}L

Liver tissue showing normal hepatic plate (HP) with orderly arranged hepatocytes and portal triad (PT) as well as prominent sinusoid and steatosis

FIG. 4 (a&b): Photomicrographs liver of (GL+NL)_H and (GL+NL)_{NH} rats (X400).

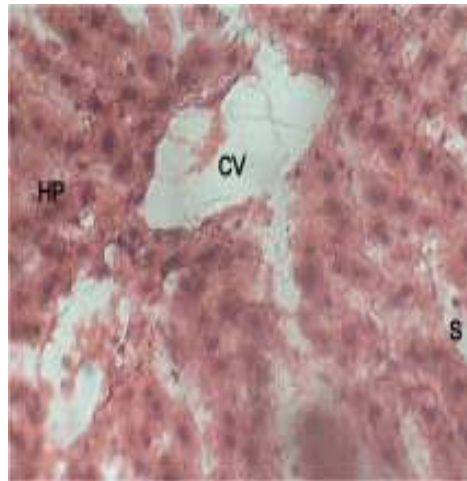
H&E= Haematoxylin and Eosin, (GL+NL)_H L = (GL+NL)_H liver, (GL+NL)_{NH} L =

(GL+NL)_{NH} liver. H=hepatotoxic rat, NH=non-hepatotoxic rat.

(GL+NL)_H = Acetaminophen (2 g/kg, bw, p.o.) + GL+NL extract (200mgkg/b.w each),

(GL+NL)_{NH} = GL+ NL extract (200mgkg-1b.w each)

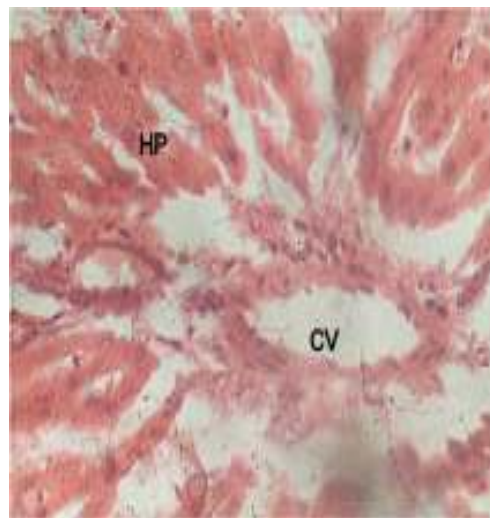
H =Hepatotoxic rats, NH = Non- Hepatotoxic rats



H&E

FIG. 5 (a) **SILYMARIN_HL**

Liver tissue with plates of hepatocytes (HP) radiating from the central vein (CV) and showing prominent sinusoids (S).



H&E

FIG.5 (b) **SILYMARIN_{NH}L**

Normal liver architecture showing central vein (CV) with normal hepatic plate (HP).

FIG. 5 (a&b): Photomicrographs liver of **SILYMARIN_H** and **SILYMARIN_{NH}** rats (X400).

H&E=Haematoxilin and Eosin, SILYMARIN_HL =SILYMARIN_H liver,

SILYMARIN_{NH}L = SILYMARIN_{NH} liver. H=hepatotoxic rat, NH=non-hepatotoxic rat.

SILYMARIN_H = Acetaminophen (2g/kg,bw,p.o.) + Silymarin commercial drug (100mg/kg, bw, p.o.)

SILYMARIN_{NH} = Silymarin commercial drug (100mg/kg, bw, p.o.)

H =Hepatotoxic rats, NH = Non- Hepatotoxic rats

4. CONCLUSION

In conclusion, the results of this study demonstrate that GL and NL have a potent hepatoprotective action upon acetaminophen-induced oxidative stress and liver toxicity in rat. The hepatoprotective effect of both extract can be correlated directly with their ability to reduce activity of serum enzymes and enhance antioxidant defiance status. The findings of this study suggest that because the intervention with the extracts in combination produces a positive synergy in hepatotoxicity amelioration relative to only GL or NL, thereby showing the strongest modulatory effect against the hepatocellular damage induced by acetaminophen, the combined extracts of *Gongronema latifolium* and *Nauclea latifolia* may be used as a safe, cheap, and effective alternative chemopreventive and protective agent in the management of liver diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. 'Author Effiong Grace' study's design and coordination, experimentation and acquisition of data, preparation of the draft and final manuscript. 'Author Mbagwu Henry' managed the literature searches, extraction and fractionation Methodologies. 'Author Essien Grace' experimentation, acquisition of data and management of the study's analysis. 'Author Udo Nsikan' technical assistance and managed the analysis of the study. 'Author Akpan Henry and Asanga Edet' graphics, analysis and interpretation of data. 'Author Eyong Ubana' experimental design, protocols and interpretation of data. 'Author Ebong Patrick' project conception and design, coordination and interpretation of data. All authors read and approved the final manuscript.

CONSENT (WHERE EVER APPLICABLE)

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

REFERENCES

1. Atangwho IJ. Biochemical impact of combined administration of extracts of *Vernonia amygdalina* and *Azadirachta indica* leaves on STZ diabetic rat models. A Ph D thesis, University of Calabar, Nigeria. 2008.
2. Bussmann RW. Sharon D. Traditional plant use in Northern Peru: Tracking two thousand years of health culture. *J Ethnobiology and Ethnomedicine* 2006; 2:47.
3. Traditional Medicine. World Health Organisation (W.H.O). 2003; Accessed 9th June, 2010. Available: www.who.int/mediacentre/factsheets/fs134/en/
4. Vickers EA. How should we research unconventional therapies? Panel report from the conference on Complementary Therapies and Alternative Medicine, National Institute of Health, U.S.A. *Int J Tech Assessment in Health Care* 1997; 13:111 – 121.
5. Nwanjo HU, Okafor MC, Oze GO. Anti-lipid Peroxidative Activity of *Gongronema latifolium* in Streptozotocin-induced Diabetic Rats. *Nig J Phy Sci* 2006; 21 (1-2):61-65.
6. Akuodor GC, Idris-Usman MS, Mbah CC, Megwas UA., Akpan JL, Ugwu TC, Okoroafor DO, Osunkwo UA. Studies on anti-ulcer, analgesic and antipyretic properties of the ethanolic leaf extract of *Gongronema latifolium* in rodents. *Afr J Biotech* 2010 5: 2316-2321.
7. Oye GI. Studies on antimalarial action of *Gryptolepis sanguinolenta* extract, *Proc Int Symp On East-West Med.* Seoul, Korea 1990; 243-251.
8. Kokwaro JO. Medicinal plants of East Africa. East Africa Literature bureau: University of Nairobi Press 3rd ed. 1976; 35.
9. Akubue PI, Mittal GC. Clinical evaluation of a traditional herbal practice in Nigeria: Preliminary report. *J Ethnopharm* 1982; 6 (3): 355-359.
10. Nworgu ZAM, Onwukaeme DN, Afolayan AJ, Ameachina FC Ayinde BA. Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. *Afri J Pharm Pharmacol* 2008; 2(2): 037-041.
11. Masubuchi Y, Suda C, Horie T. Involvement of mitochondrial permeability transition in acetaminophen induced liver injury in mice. *J Hep* 2005; 42: 110-116.
12. Kaplovitz N. Idiosyncratic drug hepatotoxicity. *Nature Reviews* 2005; 4:489-499.
13. Messner MP, Brissot P. Traditional management of liver disorders. *Drugs*. 1990; 40: 45-57.
14. Flora K., Hahn M, Rosen H, Benner K. Milk thistle (*Silybum marianum*) for the therapy of liver disease. *Am J Gastroent* 1998; 93: 139-143.
15. Skottova N, Vecera R, Urbanek K., Vana P, Walterova D, Cvak L. Effects of polyphenolic fraction of silymarin on lipoprotein profile in rats fed cholesterol-rich diets. *Pharm Res* 2003; 47:17-26.
16. Ebong PE, Atangwho IJ, Eyong EU, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants, *Azadirachta indica* and *Vernonia amygdalina* (Del). *Am J Biochem Biotechnol* 2008; 4(3):239-244.
17. Johnkennedy N Emejulu A. The protective role of *Gongronema latifolium* in acetaminophen induced hepatic toxicity in Wistar rats. *Asian Pac J Trop Biomed* 2011; S151-S154.
18. Ugochukwu NH, Babady NE, Cobourne MI, Gasset SR. The effect of *Gongronema latifolium* extract on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *J Biosci* 2003; 28 (1):1-5.
19. Etim OE, Akpan EJ, Usoh IF. Hepatotoxicity of carbontetrachloride: protective effect of *Gongronema latifolium*. *Pak J Pharm Sci* 2008; 21(3):268-274.
20. Effiong GS, Udoh IE, Mbagwu HOC, Ekpe IP, Asuquo EN, Atangwho IJ Ebong PE. Acute and chronic toxicity studies of the ethanol leaf extract of *Gongronema latifolium*. *Inter Res J Biochem and Bioinfor* 2012; 2(7):155-161.
21. Harbone JB. (1998). Methods of extraction and isolation. In: *Phytochemical methods*, Chapman and Hall, London, p. 60- 66.

22. Obadoni BO, Ochukko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Glob J Pure and Appl Sci* 2001; 6:203-208.
23. Okwu DE. Phytochemical and vitamins content of indigenous species of South Eastern Nigeria. *J Sust Agric Envir* 2004; p. 30-36.
24. A.O.A.C. Official methods of analysis Association of Official Analytical Chemist. 2; 15th ed. Washinton, D .C. U.S.A. 1990; p. 69-88.
25. Trease G E, Evans WC. *Pharmacognosy* 4th ed. USA: W.B. Saunders 1996.
26. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvic transaminases. *American Journal of Clinical Pathology* 1957; 28: 56-63.
27. Mallay HT, Evelyn KA. Estimation of serum bilirubin level with the photoelectric colorimeter. *J Biol Chem* 1937; 19: 481-484.
28. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-Phenol reagent. *J Biol Chem* 1951; 193: 265-275.
29. Nebot C, Moutet M, Hulet P, Xu JZ, Yadan J, Chaudiere J. Spectrophotometer Assay of superoxide Dismutase Activity Based on the Activated Autoxidation of a Tetracyclic Catechol *Anal Biochem* 1993; 214: 442-451.
30. Aebi H. Catalase in vitro. *Methods in Enzymology* 1984; 105:121-126.
31. Paglia DE, Valentine WN. Colorimetric assay for cellular glutathione peroxidase. *J Lab Clin Med* 1967; 70:158-169.
32. Conn, H. J. (1946). *Biological Stains: A handbook on the nature and uses of the dyes employed in the biological laboratory*. 5th edition, Geneva, N.Y., Biotech publication, pg 24-29.
33. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE. Comparative chemical composition of leaves of some anti-diabetic medicinal plants: *Azadirachta indica*, *Vernnonia amygdalina* and *Gongronema latifolium*. *Afric J Biotech* 2009; 8(18): 4685-4689.
34. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II. *J Ethnopharm* 2003; 89: 217-219.
35. Roy CK, Kamath J, Azad M. Hepatoprotective activity of *Psidium guajava* L leaf extract, *Indian J Exp Biol* 2006; 44(4), 305-311.
36. Chaturvedi R., George S, John A. Preventive and protective effect of wild basil in ethanol-induced liver toxicity in rats. *British J Biomed Sci* 2007; 64: 10-12.
37. George S, Chaturvedi P. Protective role of *Ocimum canum* plant extract in alcohol-induced oxidative stress in albino rats. *Brit J Biomed Sci* 2008; 65: 80-85.
38. Hahn G, Lehmann HD, Kurten M, Uebel H, Vogel G. On the pharmacology and toxicology of silymarin, an antihepatotoxic active principle from *Silybum marianum*. *Arzneim-Forsch Drug Research* 1968; 18: 698-704.
39. Perrisoud D. The development of cianidanol and 3-palmitoyl- 9(+)-Catechin as drugs for the treatment of liver diseases. In: *Plant Flavanoids in Biology and medicine: Biochemical, Pharmacological and structure- Activity Relationships*, Cody, V., E. Middleton and J.B. Harborne (Eds.). Alan R. Liss, Inc. New York 1986; p. 559-569.
40. Shinkim MB, Anderson MN. Acute toxicities of rotenone and mixed pyrethrins in mammals. *Proc. Soc. Exp. Biol. Med* 1963; 34: 135-138.
41. Lipkin R. Secondary plant metabolites. *Science/News* 1995; 14: 8-9.
42. Knight TR, Kurtz A, Bajt ML, Hinson JA, Jaeschke H. Vascular and hepatocellular peroxynitrite formation during acetaminophen-induced liver injury: Role of mitochondrial oxidant stress. *Toxicol Sci* 2001; 62: 212-220.
43. Bartlett D. Acetaminophen toxicity. *J Emergency Nursing* 2004; 30: 281-283.
44. Taju G, Jayanthia M, Abdul S. Evaluation of Hepatoprotective and Antioxidant activity of *Psidium Guajava* Leaf Extract against Acetaminophen Induced Liver Injury in Rats. *Int J Toxicol Appl Pharm* 2011; 1 (2): 13-20.
45. Jaeschke H. Oxidant stress precedes liver injury after acetaminophen in cultured mouse hepatocytes. *Toxicological Sciences* 2003; 72:10.
46. Knight TR, Ho YS, Farhood A, Jaeschke H. Peroxynitrite is a critical mediator of acetaminophen-hepatotoxicity in murine livers: protection by glutathione. *J Pharm Exp Ther* 2002; 303: 468-475.
47. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. *Indian J Exp Bio* 2002; 40: 765- 773.