

# Evaluation of the Oxidative Stress Status of Albino Rats Fed with Compounded Spices

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**ABSTRACT**— *In this study, the effect of mixed spiced diet, Ocimum gratissium (Ncheanwu), and Gongronema latifolium (utazi) on the oxidative stress status of albino rats was evaluated using a total of forty eight apparently healthy adult rats of both sexes, mean body weight 135.18 ± 11.25g. The rats were divided into four groups of twelve rats representing three diet groups and a control. Feeding was done twice daily for a period of three months, while analysis was done every two weeks throughout the period of the study. All the rats were provided with water and fed ad libitum. Parameters determined include malonylaldehyde oxidative stress index, antioxidant enzymes and vitamins, peroxidase activity and vitamins C and E respectively, and also glutathione another antioxidant in the cell. Result showed that the serum levels of peroxidase, glutathione and vitamins C and E significantly ( $P \leq 0.05$ ) increased in all the diet groups while the serum levels of malonylaldehyde rather decreased significantly ( $P \leq 0.05$ ) also in all the diet groups as compared with the control group. Therefore, the spices individually or in combined form have the potential to combat the oxidative stress effect and possible complications in the animal system.*

**Keywords**--- Mixed Spiced Diet; Oxidative Stress; Nutritional Therapy; Rats

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## 1. INTRODUCTION

*Gongronema latifolium* (utazi) belongs to the family of Asclepiadaceae, a climber, and an edible rain forest plant (Okafor, 1993). *G. latifolium* serves dual purpose as a spicing agent as well as medicinal herb. Phytochemical analysis showed that alkaloids are present at highest concentration (9.40mg/ml) followed by tannins, saponins, flavanoids, and sterol, (Nwachukwu, 2002; Nwachukwu and Ukoha, 2003). As a spicing agent, it serves for flavoring meat preparation and fresh “pepper soup” (Morebise et al, 2002).

Ugochukwu et al (2003), reported the hypoglycemic, hypolipidemic, and antioxidant effects. They reported reduction of malonylaldehyde level and an increase in superoxide dismutase and glutathione peroxidase activities in the blood after administration.

*Ocimum gratissium* (Ncheanwu) is commonly called wild basil and belongs to the family of Lamiaceae with characteristic aromatic smell (Iwu, 1993). The plant grows up to one meter high with dense branches and simple leaves that range from green to greenish brown (Iwu, 1993). *O. gratissium* has been found to be rich in volatile aromatic oil which plays important role in its use as a seasoning agent (Tapsell et al, 2006). In folk medicine, it has been used in the treatment of malaria, convulsion, cough, bacterial and fungal infections (Ezekwesili et al, 2004). Chaturvedi et al (2007), reported that the extract of *O.gratissium* increased ascorbic acid and glutathione levels, and decreased transaminase activities after administration. Thiobarbituric acid reactive substance level also was reduced indicating decreased lipid peroxidation by free radicals.

Reactive oxygen species (ROS) are regularly generated from everyday normal aerobic metabolism. However, with sufficient antioxidants like glutathione, vitamins C, E and antioxidant enzymes like peroxidase and superoxide dismutase (SOD) the reactive oxygen species are mopped up, thereby preventing the development of oxidative stress in the cell.

Similarly, in a state of deficient antioxidants, excess ROS leads to oxidative stress with resultant several damaging effects on cellular macromolecules many of which are deleterious (Howard et al, 2007, Browning and Horton, 2004). In this study, we evaluated the effect of *G. latifolium* and *O. gratissium* singularly and in combined form on the oxidative stress status of albino rats.

## 2. MATERIALS AND METHODS

**Materials and processing:** Fresh leaves of *Gongronema latifolium* (utazi), and *Ocimum gratissium* (Ncheanwu) were purchased from Nsukka central Market, Nsukka Enugu state. They were certified by a taxonomist from the Department of Botany, university of Nigeria Nsukka. Fresh and good leaves were sorted out and oven dried to constant weight before grinding with a warring blender to fine powdered form. This was further sieved with 1mm sieve and used to formulate Experimental feed as shown below.

Table 1. Feed Composition

Groups	Normal Rat chow(g)	<i>O.gratissium</i> (g)	<i>G.latifolium</i> (g)	Total composition
i	100.00	-	-	100.00
ii	70.00	30.00	-	100.00
iii	70.00	-	30.00	100.00
iv	70.00	15.00	15.00	100.00

The normal chow and spice samples were measured as shown , mixed, and pelleted. The pelleted feed formulation was fed to the animals to avoid wastage

## 3. ANIMAL AND TREATMENT

A total of forty eight (48) adult albino rats both sexes mean body weight  $135.18 \pm 11.25$ g were bought from Veterinary Department of University of Nigeria Nsukka Enugu State. They were divided into four groups of twelve rats and allowed to acclimatize for one week under normal rat feed and water ad libitum. The four groups represented the diet groups as shown above. After acclimatization, the rats were fed with experimental diet with water twice daily for three (3) months with the first group serving as control (received no samples)

**Preparation of Serum:** Two rats were selected from each diet group two weeks and sacrificed eighteen hours after the last feeding using light chloroform anesthesia. The thoracic cavity was carefully cut open using sterilized surgical scissors and 5.0ml of blood pipetted into a test tube with sterilized syringe and allowed to stand for 30min clotting time. It was then centrifuged at 8000xg for 10min. and the supernatant collected and used for analysis.

### Determination of Antioxidants:

The following antioxidants, vitamins C and E and glutathione were determined spectrophotometrically according to Wallin et al, (1993).

**Determination of malonylaldehyde:** This was done using thiobarbituric acid (TBA) method according to Wallin et al,(1993).

**Determination of Serum Peroxidase Activity:** The activity of serum peroxidase activity was determined by the method of Bergmeyer, (1974)

**Statistical Analysis:** All data were expressed as mean  $\pm$  SD 3 determinations and ANOVA was used to analysis variance. Values were compared to control and  $P \leq 0.05$  was regarded as significant (Woodson, 1987).

## 4. RESULTS

In table 2, the serum levels of glutathione (GSH),ascorbic acid, and vitamin E decreased significantly ( $P \leq 0.05$ ) after feeding the animals for two weeks with the spiced diet in all the diet groups as compared with the control. The result also showed that group (ii) diet (*O. gratissium* alone) exerted the highest increase in GSH, Ascorbic acid, and peroxidase activity levels. The least value of MDA was also recorded in diet group (iii), *G. latifolium* followed by diet group (ii). Chronic studies lasting for a period of three months, and parameters determined at two weeks interval (fig.i-iv) also showed that the serum values of these parameters increased significantly in all the diet groups throughout the period of

study. However, malonyldehyde (MDA) serum levels decreased significantly ( $p \leq 0.05$ ) as compared with the control diet groups, and even during the chronic study the same reduction was maintained.

The activity of serum peroxidase decreased significantly ( $p \leq 0.05$ ) as compared with the control in all the diet groups up to the 6<sup>th</sup> week (fig iv) of the study before increasing throughout the period.

## 5. DISCUSSION

The study shows that the levels of the antioxidant (Ascorbic acid, vitamin E, and glutathione) increased significantly ( $p \leq 0.05$ ) in all the diet groups while malonyldehyde level, an index of lipid peroxidation and hence oxidative stress decreased in all the diet groups as compared with the control. It was also found that the combined spiced diet group has a greater increase in the levels of these antioxidant values than the single spiced diets.

Spices are known to contain several antioxidant like polyphenols, flavanoids,  $\beta$ -carotene which may act to potentiate the activities of antioxidants in the body (Vitamins C, E and GSH) in fighting against the effect of oxidative stress (Halvorsen, et al, 2006; Tapsell et al, 2006). Vitamin C is a water soluble extracellular antioxidant which readily oxidizes to dehydroascorbic acid. The antioxidant mechanism depends on the easy to participate in oxidation reduction reaction. Vitamin C is also a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine, and neurotransmitters. Hydroxylation of aromatic drugs and carcinogens by hepatic cytochrome P<sub>450</sub> is believed to be enhanced by reducing agents like Vitamin C (Taso and Akhtar, 2005). Serum Vitamin C has been shown to decrease the risk of colon, breast, and stomach cancers (Kontush and Schekatolina, 2004). Vitamin E is the major lipid soluble antioxidant present in all cellular membranes and functions in protecting membranes against lipid peroxidation. It is known to be an important chain breaking antioxidant. It plays role in the repair of oxidative damage of membranes and DNA (Kontush and Schekatolina, 2004). It can also directly scavenge reactive oxygen molecules. Alpha tocopherol isomer of Vitamin E has been shown to protect low density lipoprotein (LDL) from oxidation (Traber, 1997).

Oxidative stress may be generated as a result of (i) Decreased blood protein content (ii) impaired glucose metabolism (iii) Genetic diseases (iv) Excessive concentration of protein (Howard et al, 2007). Excessive degradation of protein generates more than enough acetylCO<sup>A</sup> leading to increased lipogenesis, lipid peroxidation, malonyldehyde and reactive oxygen specie formation, (Osman et al, 2007).

Malonyldehyde unlike other prooxidants is very reactive and can diffuse to and through distant cells causing serious oxidative damage (Wu and Cederbaum, 2005). Therefore, the increase in the levels of the antioxidants as a result of the spiced diets suggest reduced reactive oxygen specie formation, lipid peroxidation and hence decreased risk of oxidative stress damage (Fu- et al, 1999).

In summary, the study has shown the pro-antioxidant effect of the spices studied as an individual spices and in combined form. Therefore, the presence of these spices in human diet can help to combat the effect of any oxidative stress damage as a result of generation of reactive species in the human system. The study also shows that it is better to use the spices in a combined form to get a better pro antioxidant effect.

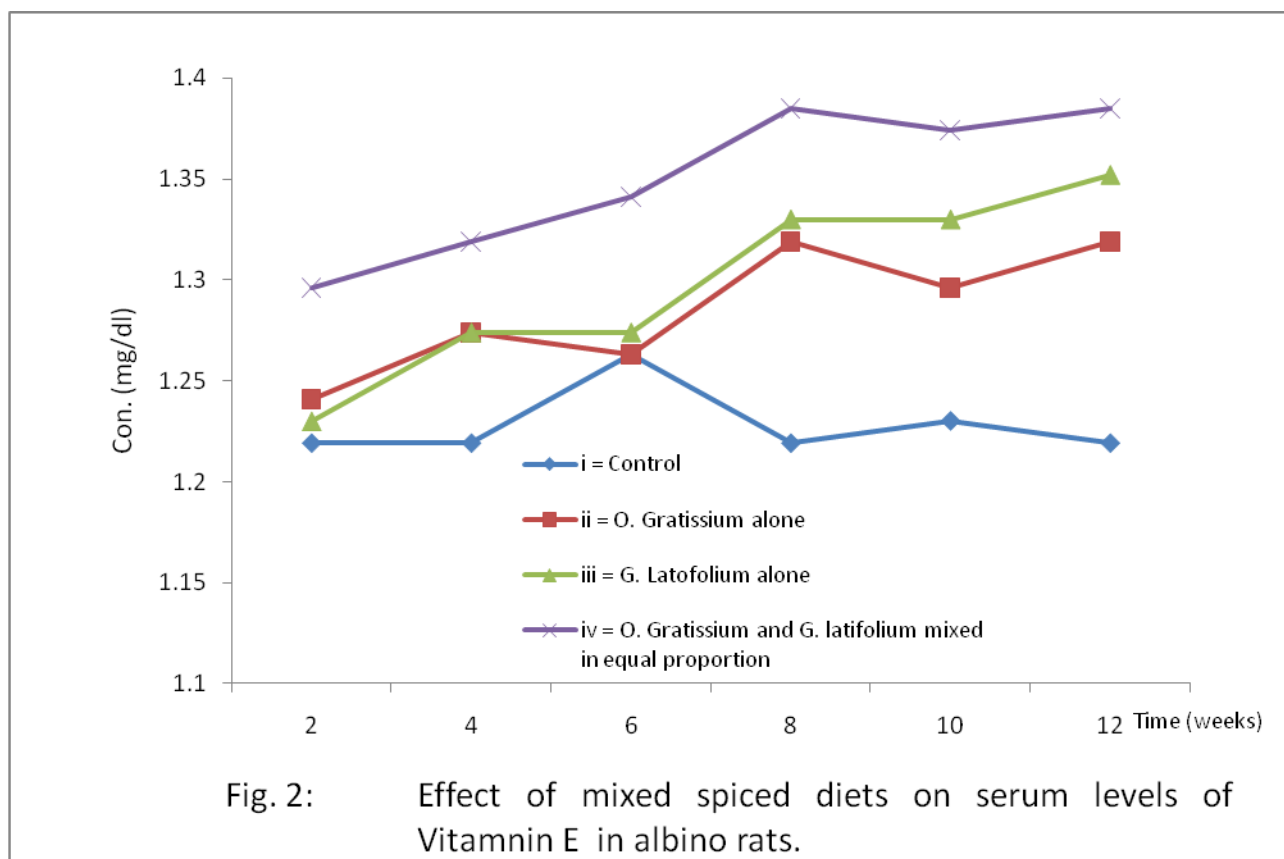
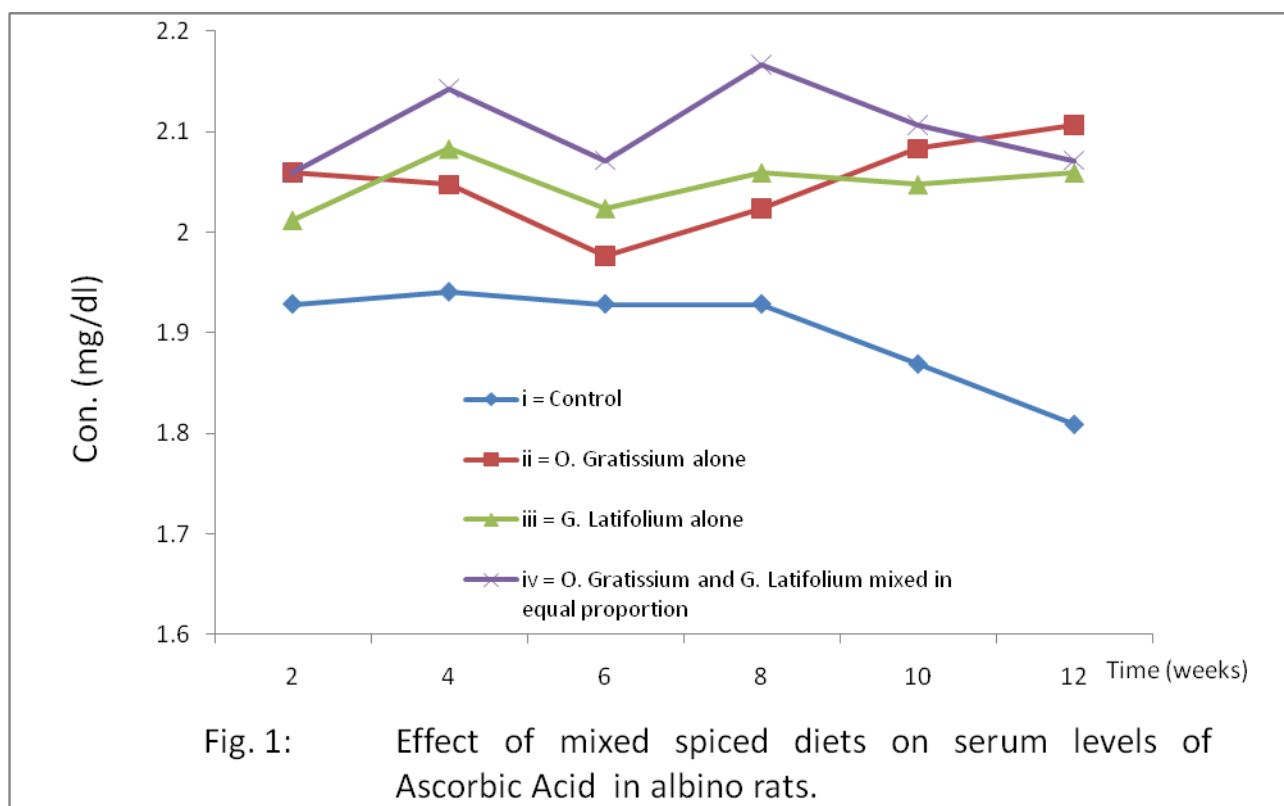
**Table 2: Effect of the spices on the oxidative indices in Rats.**

Grp	GSH( $\mu$ g/g).	Ascorbic Acid(mg/dl).	Vit. E(mg/dl).	MDA( $\mu$ g/g).	Peroxidase Activity (iu/L)
I	20.92 $\pm$ 0.21	1.92 $\pm$ 0.08	1.21 $\pm$ 0.01	0.93 $\pm$ 0.01	80.30 $\pm$ 2.10
Ii	21.02* $\pm$ 0.14	2.05* $\pm$ 0.16	1.24 $\pm$ 0.02	0.89* $\pm$ 0.02	78.80* $\pm$ 1.05
Iii	20.92 $\pm$ 0.28	2.01* $\pm$ 0.16	1.23 $\pm$ 0.02	0.91 $\pm$ 0.03	78.30* $\pm$ 1.75
Iv	20.72* $\pm$ 0.28	2.05* $\pm$ 0.50	1.29* $\pm$ 0.01	0.87* $\pm$ 0.01	76.35* $\pm$ 0.70

Feeding was done for two weeks after acclimatization.

\* Significant values as compared to control with  $p \leq 0.05$

All values are mean  $\pm$ SEM.



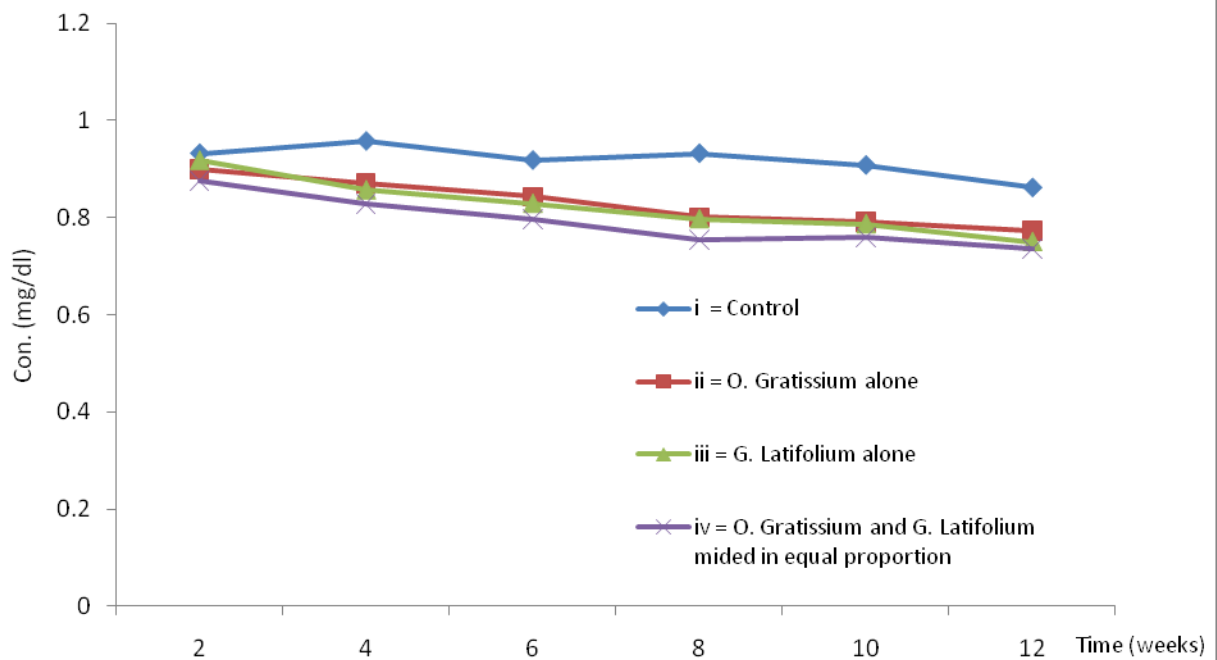


Fig. 3: Effect of mixed spiced diets on serum levels of Malondialdehyde (MDA) in albino rats.

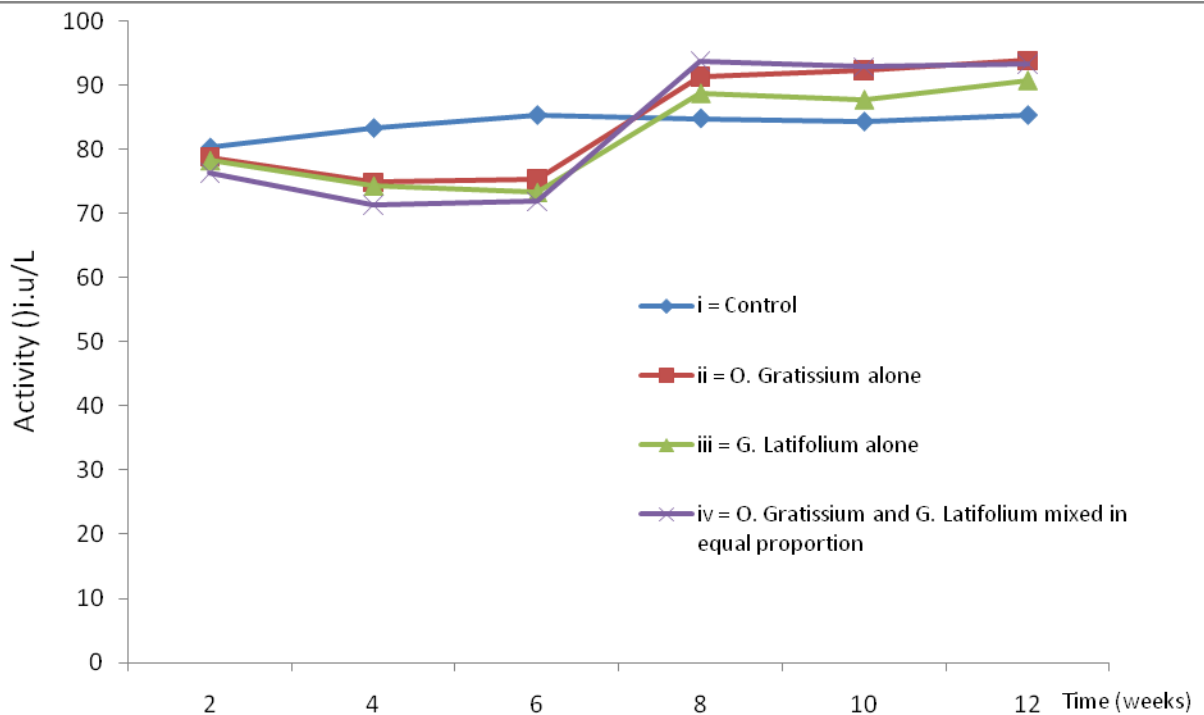
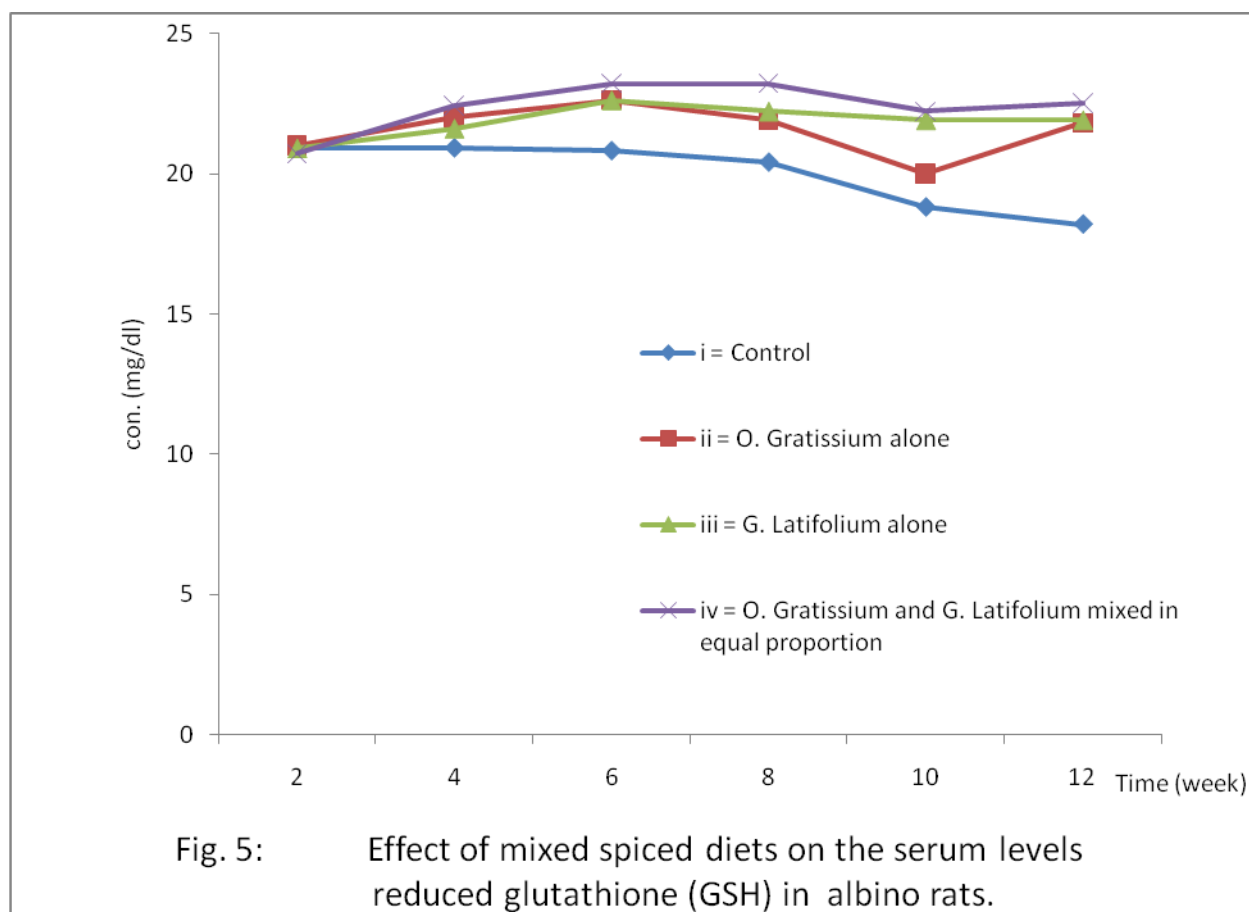


Fig. 4: Effect of mixed spiced diet on serum levels of peroxidase in albino rats.



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