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Research article

Effect of methanolic leaf extract of *Thymus vulgaris* on some biomarker enzymes in *Trypanosoma brucei* infected rats

Oluwatosin K.Shittu*, Habibat, Umar, Usman Y.Usman

Trypanosomosis Research Unit, Department of Biochemistry, Federal University of Technology, PMB65, Minna, Nigeria

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ABSTRACT

The effect of methanolic extract of *Thymus vulgaris* on some biomarker enzymes in *Trypanosoma brucei* infected rats was investigated. The result obtained in serum and liver GGT, shows that at $p<0.05$, there was insignificant difference in the serum and liver specific activities of infected untreated and uninfected treated with extract group in comparison with the uninfected untreated (normal) groups. But, there was significant increase in the serum and liver GGT activities of prophylactic treated group compared to other experimental groups. Also, the result also shows that there is significant increase in the serum ALP activities of infected early treated groups, compared to other experimental groups. However, the liver ALP activity was significantly increased in prophylactic infected treated group compared to other experimental groups. In the transaminase, the result of the serum Aspartate transaminase (AST) shows that there was significant increase in uninfected treated extract compared to other experimental groups. Whereas, there was significant increase in liver AST of infected early treated compared to prophylactic treated, infected untreated and uninfected not treated (normal) groups. However, the result of the antioxidant enzyme, Superoxide dismutase (SOD) show significant increase in serum and liver activities and liver Catalase (CAT) activities of all the experimental groups compared to uninfected not treated (normal) group. The changes in the serum and liver specific activities of these biomarker enzymes may be due to interaction between infective condition and the constituents of the extracts. Hence, caution should be taken when using the extract for therapeutic purpose because it may also have some liver or tissue membrane damaging effect.

Key words: Thymus vulgaris, *Trypanosoma brucei*, Trypanosomiasis, Biomarker enzyme, Methanolic extract

1. INTRODUCTION

Trypanosomiasis has been reported to affect humans and domestic animals. It is described as a complex debilitating and often-fatal condition caused by infection with one or more of the pathogenic tsetse-transmitted protozoan parasites of the genus *Trypanosoma* [1]. Over four decades ago, the disease, along with malaria, cancer and heart diseases, was considered by the World Health Organization (WHO) as being among the ten major health problems facing mankind. From all indications, the disease has been a great challenge to the livestock and human where the barrier imposed has been difficult to surmount by any form of chemotherapy, prophylaxis or control strategies [2]. Therefore, the need for new drug lead and target are widely acknowledge.

Thymus vulgaris L. (thyme) is an aromatic plant belonging to the Lamiaceae family, used for medicinal and spice purposes almost everywhere in the world [3]. *Thymus* genus contains one cultivated species as aromatic plant (*Thymus vulgaris* L.) and other 18 wild species [4]. *Thymus vulgaris* shows a polymorphic variation in monoterpene production, the presence of intraspecific chemotype variation is common in the genus *Thymus*. Each of the six chemotypes, geraniol (G), α-terpineol (A), thuyanol-4 (U), linalool (L), carvacrol (C), and thymol (T), is named after its dominant monoterpene [5].

Many pharmacological *in vitro* experiments carried out during the last decades revealed well defined pharmacological activities of both, the thyme essential oil and the plant extracts. The medicinal use of thyme is worthy

*Corresponding Author. Tel: +234 8033883658 Fax:
Email: toscue@yahoo.com

of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Thyme essential oil constitutes raw material in perfumery and cosmetics due to a special and characteristic aroma [6].

Also, the essential oil is well recognized for its medicinal properties in the treatment of bronchitis, whooping cough and tooth ache. The herb or its infusion is also given for several disorders. It is possible that the flavonoids present may be important, such as in the spasmolytic activity of the smooth muscles of the guinea pig ileum and trachea. It was also found that essential oil; thymol and carvacrol, have antimicrobial activity against fungi (some aflatoxins producers), viruses, helmiths, gram positive bacteria (including botulinum) and gram negative bacteria [7]. However, we have earlier reported that the administration of methanolic leaf extract *thymus vulgaris* at 500mg/kg body weight to *Trypanosoma brucei*-infected rats was able to reduce the replication of parasite of infected treated rats when compared with infected untreated rats and ameliorate the haematological effect caused by *T. brucei* infection on some haematologicals. Therefore, in this study, the effect of 500 mg kg body weight of methanolic leaf extract of *Thymus vulgaris* was investigated on some biomarker enzyme.

2. MATERIALS AND METHODS

2.1. Plant material collection

Fresh leaves of *thymus vulgaris* commonly called thyme leave were collected from Kure central market, Minna, Niger State, North central Nigeria on April/May 2012. 2012 and authentication was carried out at Department of Plant biology, Federal University of Technology, Minna, Niger state.

2.2. Preparation of plant extract

Fresh leaves of the plant were dried at room temperature and pulverized to powder using an electric blender, 200g of the powder was percolated in 1600ml of absolute methanol and kept in shade for 48hours after which it was filtered [8]. The solvent was removed from the filtrate using rotary evaporator.

2.3. Parasite strain

Trypanosomal brucei brucei was obtained from the Nigeria institute for Trypanosomiasis research (NITR) Vom, Plateau state/Nigeria and maintained in the laboratory by serial blood passage in rats until required.

2.4. Experimental animals

Healthy albino rat of average weight 120-150g were purchased from Animal House, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Kaduna state. The rats were housed in plastic cages and maintained under standard laboratory conditions in the biochemistry laboratory, Federal University of Technology Minna till they reached the desired weight. They have free access to rat pellets and tap water *ad-libitum*.

2.5. Trypanomes

Trypanosome brucei brucei infected blood was obtained from the tail of infected rats at high parasitemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated into the peritoneal cavity of uninfected rat weighing approximately 250g. The suspension as described earlier [9,10] contained 3 or 4 trypanosomes per view at x100 magnification (approximately 106 cells per mL).

2.6. Experimental design

Twenty – five (25) rats were used in this experiment and divided into five (5) groups as follows.
Group 1- Not infected not treated (Positive control)
Group 2- Infected not treated (Negative control)
Group 3- Infected prophylactic treated extract
Group 4- Infected early treated extract
Group 5- Not infected treated extract
The serum and liver were obtained from each rat at day 6 post infection.

2.7. Parasite count

Blood smear obtained from the tail of the infected rats and the number of parasites was determined microscopically by counting under the microscope at x40 magnification on a daily basis.

2.8. Collection of blood and liver

The animals were anasthesized with chloroform and blood was collected through cardiac puncture into the centrifuge tube and centrifuged at low speed for 15minutes to get the serum. The rat was dissected to reveal the internal organ and the liver was removed and placed in sample bottles containing sucrose solution (0.25mL) to maintain a normal physiological environment. The liver was homogenized and the supernatant were stored in sample bottles for subsequent used.

2.9. Enzyme and protein determination

All enzyme assay kits were products of Randox Laboratories Ltd, United Kingdom. Total protein concentration was determined using Biuret method described by [11] as modified by [12]. Alkaline phosphatase (ALP) was

determined based on the method of [13], Gamma glutamyl trasferase was assayed using the method described by [14] and Aspartate transaminase (AST) and alanine transaminase (ALT) activities was assayed using the method described by [15]. Also, Catalase (CAT) activity was determined as described by [16] and superoxide dismutase (SOD) activities was determined by the method of [17] which was based on the ability of superoxide dismutase to inhibit the reduction of nitroblue tetrazolium by superoxide.

2.10. Ethical clearance

Ethical clearance was given by the Institutional Review Board of the Nigeria institute for Trypanosomiasis research (NITR) Vom, Plateau state/Nigeria and Department of Biochemistry, Federal University of Technology, Minna/Nigeria Ethical Review Board (CUERB) in accordance with International Standard on the care and use of experimental animals.

2.11. Statistical analysis

The mean ± SEM and significant difference between means evaluated by analysis of variance (ANOVA). Post test analysis was done using the Duncan Multiple Comparison tests. Values of $p<0.05$ were considered as statistically significant [18].

3. RESULTS

3.1 Alkaline phosphatase

At $p<0.05$, the result shows that there is no significant difference in the serum ALP activities of infected untreated and uninfected treated with extract compared to the uninfected untreated (normal) group. Whereas, there was significant increase in infected early treated and infected prophylactic treated groups. Also, there was significant increase in the liver ALP activities of infected early treated, infected untreated and infected prophylactic treated compared to the uninfected untreated (normal) group (Fig.1).

3.2. Gamma glutammyl transferase

At $p<0.05$, the result of the specific activities of GGT shows there was significant increase in the serum GGT of all the experimental groups compared to uninfected untreated(normal) group but serum GGT activities of prophylactic treated group was significantly higher than other groups. Also, there was no significant difference, in the liver of infected untreated and uninfected treated with extract group in comparison with the uninfected untreated (normal) group but there was significant increase in that of infected early treated and infected prophylactic treated groups in comparison with the uninfected untreated (normal) group (Fig.2).

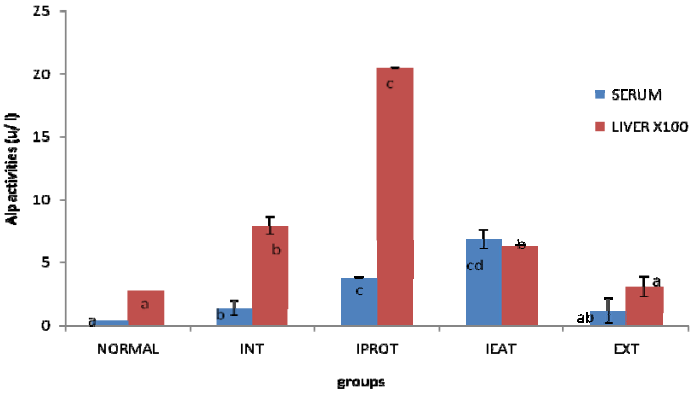

Fig.1. Specific activities of alkaline phosphatase (ALP) in serum and liver of rats infected with *T. brucei*. Results are mean of four determination ± SEM. Bars carrying different letters are significantly different at $p<0.05$.
NORMAL: Non Infected untreated, INT: Infected untreated, IPROT: Infected prophylactic treated, IEAT: Infected early treated, EXT: Uninfected treated with extract.

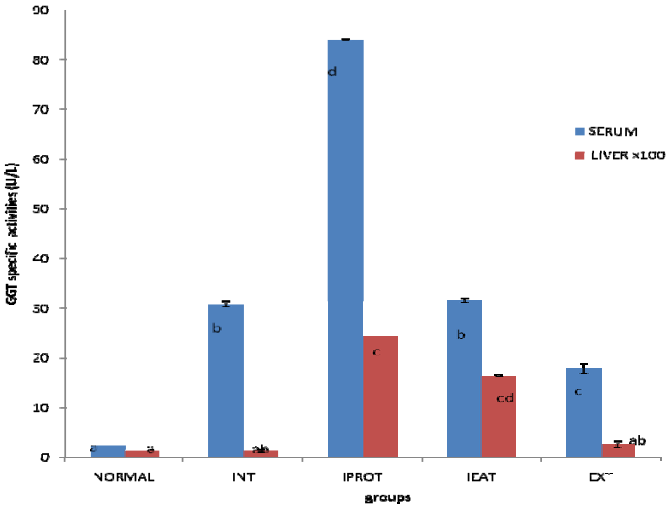

Fig.2. Specific activities of gamma glutammyl transferase (GGT) in serum and liver of rats infected with *T. brucei*. Results are mean of four determination ± SEM. Bars carrying different letters are significantly different at $p<0.05$.
NORMAL: Non Infected untreated, INT: Infected untreated, IPROT: Infected prophylactic treated, IEAT: Infected early treated, EXT: Uninfected treated with extract

3.3. Alanine transaminase (ALT)

The results of the ALT activities in the serum and liver are presented in Fig. 3. The result of the serum ALT activities showed a significant increase ($p<0.05$) in prophylactic treated infected rats and infected early treated compared to infected not treated, uninfected extract treated and uninfected untreated groups. Also, there was significant increase in ($p<0.05$) in liver ALT of all the experimental groups compared to uninfected untreated (normal) group (Fig.3).

3.4. Aspartate transaminase

The serum aspartate transaminase (AST) show significant ($p<0.05$) increase in uninfected treated extract when

compared other experimental groups. Whereas there was significant increase in liver AST all other experimental groups compared to uninfected untreated (normal) group ($p<0.05$) of all the groups (Fig.4).

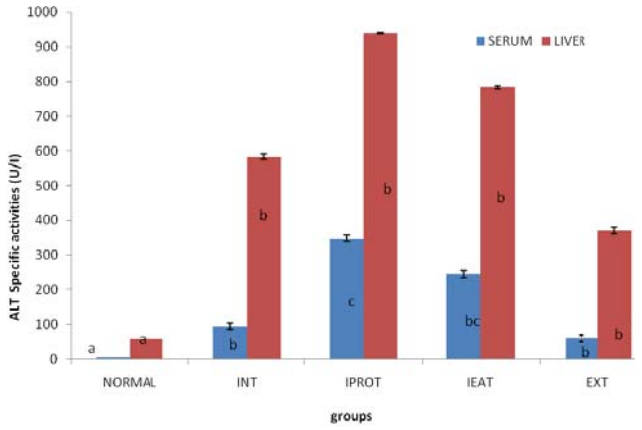

Fig.3. Specific activities of alanine transaminase (ALT) in serum and liver of rats infected with *T. brucei*. Results are mean of five determination ± SEM. Bars carrying different letters are significantly different at $p<0.05$.
NORMAL: Non Infected untreated, INT: Infected untreated, IPROT: Infected prophylactic treated, IEAT: Infected early treated, EXT: Uninfected treated with extract

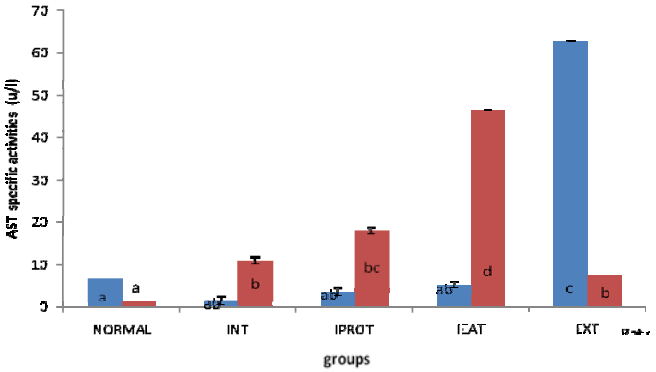

Fig.4. Specific activities of aspartate transaminase (ALT) in serum and liver of rats infected with *T. brucei*. Results are mean of five determination ± SEM. Bars carrying different letters are significantly different at $p<0.05$.
NORMAL: Non Infected untreated, INT: Infected untreated, IPROT: Infected prophylactic treated, IEAT: Infected early treated, EXT: Uninfected treated with extract.

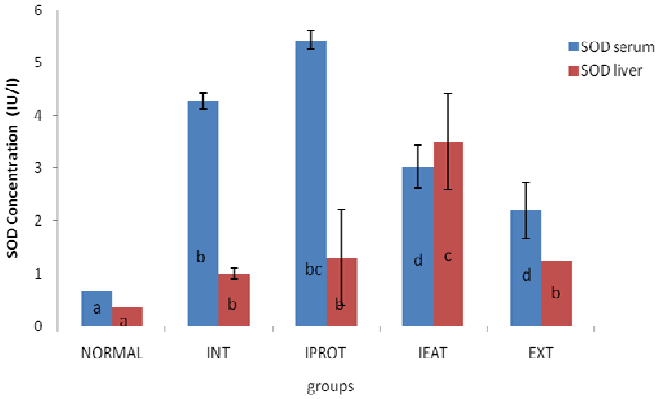

Fig.5. Specific activities of superoxide dismutase (SOD) in serum and liver of rats infected with *T. brucei*. Results are mean of four determination ± SEM. Bars carrying different letters are significantly different at $p<0.05$.
NORMAL: Non Infected untreated, INT: Infected untreated, IPROT: Infected prophylactic treated, IEAT: Infected early treated, EXT: Uninfected treated with extract.

3.5. Superoxide dismutase (SOD)

The specific activities of superoxide dismutase (SOD) in the serum and liver are shown in Fig.5. There was significant increase ($p<0.05$) in serum superoxide dismutase (SOD) of all other experimental groups compared to the uninfected not treated (Normal) groups. Also, there was significant increase ($p<0.05$) in liver SOD of infected early treated compared to infected untreated, prophylactic infected treated and infected extract treated groups.

3.6. Catalase (CAT)

The specific activities of catalase (CAT) in serum of all the experimental groups shows significant increase ($p<0.05$) compared to the uninfected untreated (normal) while there was significant increase in liver CAT activities of prophylactic infected treated compared to infected not treated, infected early treated and uninfected extract treated groups which show no significant difference at $p<0.05$ (Fig.6).

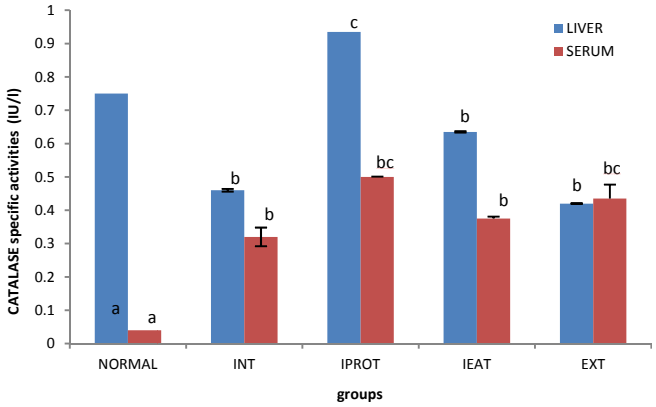

Fig.6. Specific activities of catalase in serum and liver of rats infected with *T. brucei*. Results are mean of four determination ± SEM. Bars carrying different letters are significantly different at $p<0.05$.
NORMAL: Non Infected untreated, INT: Infected untreated, IPROT: Infected prophylactic treated, IEAT: Infected early treated, EXT: Uninfected treated with extract

4. DISCUSSION

Previous studies have shown many tropical plants contain constituents that are clinically efficacious against many protozoal diseases [19,20] and that the trypanocidal activity of certain plant extracts are due to the alkaloids and other constituents present [21]. A number of studies have reported that thymus vulgaris has nutritional and pharmacological properties. It has been reported that the oil components; thymol and cavacrol are responsible for its analgesic, antioxidant, antispasmodic, and insect repelling properties [22].

Analysis of serum enzymes have been reported to be of value and are early warning signs for certain diseased conditions. Many enzymes are present in plasma (or serum) and their activity can be easily assayed in serum with diagnostic reagents. Alkaline phosphatase is distributed in almost every tissue of the body, especially those involved in active transport mechanism. Serum alkaline phosphatase levels are of interest in the diagnosis of hepatobiliary disorder and bone disease [23]. The significant increase ($p<0.05$) of liver ALP activities of the infected untreated, prophylactic treated and uninfected early treated (Fig.1) compared to uninfected extract treated and normal groups, may be attributed to interaction between toxin release by the parasite and the constituents of the extracts.

Gamma glutammyl transferase (GGT) is an enzyme derived from endoplasmic reticulum of the cells of the hepatobiliary tract. As this reticulum proliferates, for example in response to drugs, synthesis of the enzyme is induced and plasma GGT activity increase. Therefore a rise in plasma activity does not necessarily indicate hepatocellular damage [24].

Prophylactic treated group shows a significant increase ($p<0.05$) in serum GGT when compared to other groups (Fig.2). In the liver, there was significant decrease in infected untreated and uninfected extract treated when compared with the prophylactic treated and infected early treated groups. This implies that there was no leakage of the enzyme into serum but induction of the enzyme synthesis in the liver during the interaction between toxin release by the parasite and the active constituents of the extracts.

The transaminases (ALT and AST) activity of serum and liver are slightly increased in the infected not treated when compared with normal rats (Fig.3 and Fig.4). These suggest and probably confirm earlier results that infection could lead to gradual tissue especially liver destruction as increase in the liver activities were observed. Thymol and carvacrol that have been implicated as active component of *Thymus vulgaris* [22] might be the cytotoxic constituent.

Reactive oxygen species (ROS) are oxidants formed in our body due to exogenous and endogenous factors and are found to be responsible for many diseases such as cancer, cardiovascular disease, neurodegenerative diseases, inflammatory disease, ischemia-reperfusion injury and aging [25]. Antioxidant systems are normally put in place in living aerobic organisms to counter the effect of oxidative stress [26]. It has been reported that phytochemicals have the ability to neutralize the free radicals or reactive oxygen species responsible for the onset of the diseases [25]. The mechanisms by which the plant biomolecules provide defense against ROS are: ROS scavenging, reduction of peroxides and repair of peroxides membrane, utilization of dietary lipids and alternative biological pathways that occur in cancers, multiple system organ failure and diabetes.

Catalase is a peroxisomal marker enzyme found in blood, bone marrow, mucous membrane, kidney and liver. It functions assumed to be destruction of hydrogen peroxide. The result showed increase in the serum CAT activities in all experimental groups when compared to uninfected untreated (normal) group (Fig.2), which may be attributed to induction of the enzyme in the presence of reactive metabolite from the extract and disease condition. Superoxide dismutase (SOD) is also an antioxidant enzyme that protects blood cells from oxidative stress and damage. It is stimulated by contact with micro organism and neutrophil exhibit respiratory burst. The result of this work showed that serum and liver SOD activities increased significantly in other experiment groups when compared to uninfected untreated (normal) group (Fig.6). This could be indication of its continuous utilization as a scavenger of reactive oxygen species (ROS).

5. CONCLUSIONS

In conclusion, it can be suggested that *Thymus vulgaris* methanolic leaves extract has trypanocidal properties and increases the serum and liver specific activities of some biomarkers, which might be due to interaction between infective condition and the constituents of the extracts. Hence, caution should be taken, when using the extract for therapeutic purpose because it may also have some liver or tissue membrane damaging effect.

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