

Controlling the Influence of Biochemical Changes Induced by DMBA through the Methanolic Maceratives of Pre-pupal Stages of Black Soldier Fly, *Hermetia illucens* (L.) (MMPPSBSF) in Rats

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ABSTRACT

The preservative role of the methanolic maceratives of pre-pupal stages of black soldier fly, *Hermetia illucens* (L.) (MMPPSBSF) was assessed against the 7,12-dimethylbenz[a]anthracene (DMBA)-induced changes in biochemical parameters in blood of rats. Administration of DMBA in the experimental animals (rat, *Rattus norvegicus* L.) caused decrease in levels of total proteins, albumin and globulin; elevation of urea, uric acid and creatinine levels and elevation of the velocity of biochemical reactions catalysed by the enzyme Aspartate-Aminotransferase (AST); enzyme Alanine Aminotransferase (ALT) and enzyme Lactate dehydrogenase (LDH). Treating the experimental animals (rat, *Rattus norvegicus* L.) with Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) in present attempt was found to cause a significant increase in the levels of total proteins, albumin and globulin in the serum. Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) thus, providing protection through maintenance of level of the total proteins, albumin and globulin in the serum. Use of Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) may open a new avenue to control the damages caused by the carcinogens.

Keywords: Biochemical Parameters; 7,12-Dimethylbenz [a]anthracene or DMBA; Oxidative Stress; MMPPSBSF

Introduction

DMBA (7,12-dimethylbenz[a]anthracene) exert influence through reduction of activation of efficiency of immune system in the animals. DMBA (7,12-dimethylbenz[a]anthracene) is a polycyclic aromatic hydrocarbon (PAH) and popular to cause tumors in rats [1]. According to Demir and Demirbag (1999) [2], the petroleum and some of its derivatives are the polycyclic aromatic hydrocarbon (PAH). They are widespread organic pollutants.

Through the oil spills and incomplete combustion of fossil fuels, the polycyclic aromatic hydrocarbon (PAH) enters the environment and exert the unfavourable conditions for the life on earth. As significant member of the polycyclic aromatic hydrocarbon (PAH) group, the "7,12-dimethylbenz[a]anthracene (DMBA)" compound in the form of persistent organic pollutant exists ubiquitously in the environment. The "7,12-dimethylbenz[a]anthracene (DMBA)"

compound is mainly formed through the incomplete combustion of organic materials, such as gasoline, coal and cigarettes [3].

United Nation's Food and Agriculture Organization (FAO) [4] is a specialized and significant agency. It is leading in international efforts to defeat hunger and for the improvement in the quality of nutrition and the security of the food. The United Nation's Food and Agriculture Organization (FAO) has estimated that by 2050, the population of the world is going to reach nine-billions. It is therefore, necessary to increase the food production at least by seventy percent. The meat production should also by hundred percent (FAO, 2020) [4]. Then and then only, it may be possible to meet global demands. However, present agricultural practices appear to be insufficient with reference to sustainability. The importance of security of the food has been experienced by the world in the COVID-19 pandemic. During this COVID-19, many food processors and food supply chain stakeholders were shut down. This system exerted influence on creating a meat shortage and increasing food insecurity concerns. In addition, meat accounts for only fifteen percent of the total energy in the global human diet. Approximately eighty percent of agricultural land is used for grazing the animals and the production of livestock feed-fodder [5,6].

The consumption of meat must be reduced by seventy percent. This reduction in meat consumption is to achieve sustainable food production systems and meet food security requirements [7,8]. Furthermore, according to Ziolkowska (2017) [9], food loss is one more challenge for the food sustainability, food economics and the food nutritional status. Despite considerable progress in agricultural production, post-harvest practices and supply chain management, in United States, there is loss of thirty to forty percent of total food production. For the purpose to reduce wastage of available food, to increase the yield of production and for the provision of alternative sustainable protein moieties with minimum impact on environment, therefore, there is necessary to develop novel system of production of food production. According to Liceaga (2019) [10], one sustainable food system is entomophagy, or consumption insects as a food material by human being. As a part of a diet, the insect consumptions are widely followed in Asia, Africa and Latin America. Anaya, et al. [11] reported ninety five percent of the biodiversity for the insects. This figure represents the largest sector of fauna and have historically been consumed at various stages of their life cycle. In Zambia, Nigeria, and other African countries, the meat supply is insufficient. Insects are therefore, serving as a valuable source of protein in Zambia, Nigeria, and other African countries [12]. According to Van Huis [13], insects are with fifty to seventy one percent of proteins; thirteen to thirty three percent of fats and five to thirteen percent of fibres.

In addition, insects are with low emissions and greenhouse gas

production, excellent feed conversion ratios, low water consumption and inexpensive feed sources. That is to say, the insects serve as favourable candidates as alternative protein that may be developed for food and feed products. Acceptance of insects as source of food material by the human population (especially in western countries) appears to be the most important hurdle. Moreover, it is challenge for researchers. According to Borremans (2020) [14], insects may be accepted by consumers (of developed countries) when they are fragmented and included in a food as a protein powder or ingredient. Ovissipour, et al. [15] suggested application of enzymatic hydrolysis technology for protein recovery; production of a broad spectrum of food and production of feed ingredients. This method is going to produce functional food with improved and upgraded functional properties and protein nutritional value. From perspectives of a food science and technology, attempts have developed protein hydrolysates from different insects including cricket, *Grylloides sigillatus* (L.) [16]; migratory locusts, *Locusta migratoria* (L.) [17]; mealworm, *Tenebrio molitor* (L.) [18] and black soldier fly (BSF), *Hermetia illucens* (L.) [19-26].

For the antioxidant properties of black soldier fly (BSF) hydrolysates, there are reports through few attempts on the hydrolysis of black soldier fly (BSF) [20,22]. Caligiani, et al. (2018) and Mintah (2020) [19,21], reported the method of chemical and enzymatic hydrolysis of BSF for extraction and characterization of different fractions for the antioxidant properties of black soldier fly (BSF). The extractives of prepupal stages of black soldier fly (BSF) through proper solvent for the analysis of functional properties, antioxidant activity, nutritional value and protein structure have not been evaluated through a systematic approach. The present attempt aimed to evaluate the protective effect of the Methanolic Extractives of Prepupal Stages of Black Soldier Fly (MEPSBSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) through the Norwegian Rat, *Rattus norvegicus* (L) against the DMBA-induced changes by determining levels of urea, uric acid, creatinine, total protein, albumin, globulin and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) activities in blood of rats.

Methods and Material

The study was carried through the steps, which include Nurturing (Rearing) of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae); Preparation of "Methanolic Extractives of Prepupal Stages of Black Soldier Fly (MEPSBSF)"; Design of Experiment through Grouping the experimental animals; Processing for the assay sample preparation (Serum Assay Sample and Liver tissue homogenate); Biochemical Analysis and Statistical Analysis of the data.

Nurturing (Rearing) of the Black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae)

The method explained by Vitthalrao B. Khyade (2021) [26] for rearing the Black Soldier Fly, *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) in local environmental conditions of Baramati (India) was followed. The present attempt on the rearing of the black soldier fly, *Hermetia.illucens* (Linnaeus) (Diptera: Stratiomyidae) in local environmental conditions of Baramati (India) has biology of was carried during 4 November, 2020 to 28 February, 2021 (First attempt); 1 March, 2021 to 21 June, 2021 (Second attempt) and 1 July, 2021 to 3 November, 2021) in the insectary (Green House) of Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati, Pune, India. The culture was initiated through keeping household organic waste (Kitchen Waste). The content of the organic waste (Kitchen waste) was with sour milk, waste tea powder, vegetable waste (cabbage and fruits of papaya). This content of the organic waste (Kitchen waste) was taken in a box and labelled as "tray with rearing bed" (or Larval Rearing Bin). This box (Larval Rearing Bin) was designed in the shape of a rectangular wooden box with the dimensions of 2x1.5x1.5 feet with ventilation holes on the top lid, and a rectangular plank was placed at an inclined position making an angle of 45o with the bottom so as to facilitate the process of harvesting (auto-harvesting) of full grown (matured) larvae.

The fully grown (matured) larvae use to convert into next life stage (pre-pupa). Little amount of water was used to spray on the contents in a tray. Spraying the water on organic waste initiates the process of decomposition through bacteria. After a few days as the wastes began to decompose [27]. The fertilized egg mass of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) was procured from Mangal Agro Farm Miri Rd, Maka, Maharashtra 414501 India. The egg mass was kept suspended over fresh food (slices of fruits of papaya, *Carica papaya* L.). For uniform hatching, it requires a humid and cool place with fresh airflow. Hatching of the eggs take place within twenty-four of hours of provision of favourable conditions to the fertilized eggs. On fifth day after hatching, the larvae from incubation box were transferred to box with rearing bed (Larval Rearing Bin). The larvae were allowed for feeding and their development. The mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) were collected from this stock culture. The mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) were transferred to the rearing cages once in three days.

This transfer of the mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family:

Stratiomyidae) is to the rearing cages observe different life stages. The cages with the mature stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) were placed inside the insectary (Green House) of Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati, Pune, India. After three days, the stages of prepupa of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) from the rearing cages were placed in a small plastic bucket containing soil and kept inside the rearing cage in order to provide a place for pupation. The condition of humidity of the cage was maintained at 70-80 %. This was achieved through keeping a water source with sponges soaked in it as well as by spraying water three to four times a day [[27-29]. The source of lighting was provided daily for twelve hours. The light provision is to stimulate adult mating. The card boards were made hung in various locations. The provision of the card boards is to mimic sites for laying the eggs by the adult female black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) [27,30-34]. The observations were recorded on egg hatching, the period of development of the larval, pupal and adult stages and the morphology of the life stages. The sex ratio was determined through random sampling performance and observations of the genitalia of randomly collected adults.

For the purpose of determination of frequency of mating of newly emerged adult flies of the-black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae) and egg laying capacity (fecundity), the sets of experimentation were ten in number. The sets of experimentations were identical and in the form plastic containers (capacity: 2 L). Newly emerged adult male and adult female were kept in pairs plastic containers. All these setups were then placed in the insectary (Green House) of Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati, Pune, India. They were provided with artificial lighting (60W) and humidity (70-80 %) [28,29,35-38]. The observation on the determination of the egg laying sites (ovipositional sites); egg laying period (ovipositional period) and the life span was carried every twelve hours. The eggs were collected from this set up. The eggs were allowed to hatch under varying conditions. This attempt was for the purpose to determine period of incubation and the ability of the eggs to tolerate unfavourable temperatures. The eggs and larvae were collected daily from the rearing bin. Larvae were taken back to laboratory. The larvae were washed thoroughly to remove impurities. The larvae were knocked out by freezing and measured using coulometer to record total body length, body width, and length of mouth hook. The Dyar's rule was followed for the purpose of determination of the morphometry of the larvae. It was carried through the determination of number of larval instars in its life cycle of the black-soldier-fly, *Hermetia.illucens*

(Linnaeus) (Order: Diptera; Family: Stratiomyidae) [34,39-43]. The olfactometer was utilized for the determination of behaviour of the feeding and the preference of food waste by the larval stages of the black-soldier-fly, *Hermetia.illucens* (Linnaeus) (Order: Diptera; Family: Stratiomyidae). For the purpose to get the consistency for the data processing for the results, each attempt was repeated at least for three times. The collected data was subjected for analysis through the statistical method.

Preparation of Methanolic Extractives of Prepupal Stages of Black Soldier Fly (MEPSBSF)

The mature pre-pupal life stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) were selected randomly from the stock culture. They were kept in freezer at -35°C for twenty-four hours. After twenty-four hours of freezing, they were subjected for thawing followed by washing thoroughly. The content was then processed for drying for forty-eight hours in oven (60 °C). Through the use of blender, the oven dried pre-pupal stages of the Black-Soldier-Fly (BSF), *Hermetia.illucens* L. (Order: Diptera; Family: Stratiomyidae) were subjected for grinding until smooth. The content thus obtained was titled as, "Black-Soldier-Fly-Meal" (BSF Meal). For the purpose to prepare the extractives from "Black-Soldier-Fly-Meal" (BSF Meal), methanol was selected as solvent. Ten milligrams of "Black Soldier Fly Meal" (BSF Meal) were mixed in hundred millilitres of methanol. The contents were kept for twenty-four hours at room temperature for maceration. The method of obtaining extractives through the maceration belong to Choi, et al. [44]. After twenty-four hours of maceration, the content was filtered through the use of common laboratory filter paper. For the purpose to obtain extractives in concentrated form, the filtrate was subjected for evaporation. Rotary evaporator was utilized. This evaporator was with a reduced pressure and temperature of 40°C.

Rearing of Brown Rat, *Rattus norvegicus* (L), the Experimental Animals

For the present attempt on utilization of the methanolic extractives of prepupal stages of Black Soldier Fly (MEPSBSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) for treating the DMBA induced hepatotoxicity and free-radical damage in Norwegian Rat, *Rattus norvegicus* (L), fifty adult females (12 weeks-old) Brown rats (*Rattus norvegicus* L) (Dr APIS Laboratory), weighing 170 - 220 g were procured from the Department of Zoology, Savitribai Phule Pune University. The adult female rats were housed in quiet cages (20 - 25°C; 50 - 60% relative humidity). They were kept in laboratory with a condition of "12 hours light/dark cycle (7 a.m. - 7 p.m.)". They were fed with a commercial standard rat diet (Abaliogu YemSanayi, Denizli, Turkey) and water ad libitum. All animal procedures were approved by the Animal Care and Use Protocol (Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Baramati).

Design of Experiment through Grouping the Experimental Animals

Total fifty adult female brown Norwegian rat, *Rattus norvegicus* (L) (12 weeks-old) Brown rats weighing 170 - 220 g were procured from the Department of Zoology, Savitribai Phule Pune University. They were housed in quiet cages (20 - 25°C; 50 - 60% relative humidity). They were kept in laboratory with a condition of "12 hours light/dark cycle (7 a.m. - 7 p.m.)". They were fed with a commercial standard rat diet (Abaliogu Yem Sanayi, Denizli, Turkey) and water ad libitum. All animal procedures were approved by the Animal Care and Use Protocol (Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Baramati). The experimental animals were divided into four groups, each with ten individuals. Remaining ten individuals of experimental animals were maintained as reservoir. The individuals of group: first were served as untreated control group. The individuals of this untreated control group received 0.3 ml corn oil daily orally. The individuals of the group: second were served as DMBA treated group. The rats in this group: second were supplied with 7,12- Dimethylbenz [a] anthracene (DMBA). The single dosage of DMBA at the rate 335 mg/kg of body weight was selected.

The DMBA was given along with corn oil [45-47]; The individuals of group: third were served as Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) treated group. The rats in this group: third were supplied with methanolic extractives of pre-pupal stages of black soldier fly (MEPSBSF). The methanolic extractives of pre-pupal stages of black soldier fly (MEPSBSF) was given orally, 100 mg/kg/day every twenty-four hours. This MEPSBSF treatment was continued for seven days. The individuals of group: fourth were served as "DMBA + MEPSBSF treated group. The rats in this group were the recipient of single dosage of DMBA. The strength of DMBA dosage was of 335 mg/kg Body Weight. The rats in this group were also supplied with methanolic extractives of pre-pupal stages of black soldier fly (MEPSBSF). The number of dosages of methanolic extractives of pre-pupal stages of black soldier fly (MEPSBSF) were seven. Each dosage of methanolic extractives of pre-pupal stages of black soldier fly (MEPSBSF) was after every twenty-four hours. The strength of each dosage of methanolic extractives of pre-pupal stages of black soldier fly (MEPSBSF) was 100 mg/kg/day. The methanolic extractives of pre-pupal stages of black soldier fly (MEPSBSF) treatment was oral, at every twenty-four hours and it was for total seven days.

Processing for the Assay Sample Preparation

At the end of the schedule of seven days of treatment, experimental animals were anesthetized. This anaesthetization was carried through two intra-peritoneal injections. The first intraperitoneal injection was of ketamine (60 mg/kg). The second

intraperitoneal injection belong to xylazine (6 mg/kg). The gap between the two injections was fifteen minutes. Through the use of sterile tubes, the blood samples intracardiac were collected.

Biochemical Analysis

For the purpose of serum bioassay for the level of total protein, albumin, globulin, urea, creatinine, uric acid and velocity level of biochemical reactions catalysed by the enzymes (ALT; AST and LDH), the blood samples were processed for centrifugation and serum preparation. Each blood sample was allowed for centrifugation at $2000 \times G$ for 15 minutes, at $4^{\circ}C$. The serum, use to appear as the top yellow layer in centrifugation tube. This top yellow serum layer was pipetted out. Care was taken for keeping the white buffy layer (Serum was collected without disturbing the white buffy layer). The bioassay methods in present attempt include: method of Lowry, et al. (1951) [48] (for total proteins); Bromcresol Green method described by Rosenberg and Klotz (1960) [49] (for albumin); method of George (2009) [50] (for globulin); modified Berthelot method described by Misic, et al. [51] (for serum urea determination); Kinetic enzymatic method (for determining serum creatinine Moss, et al. [52]; Morin method (1974) [53] (for determining serum uric acid); method of Kori-Siakpere Ovie, et al [54] (for the bioassay of activities of Aspartate Aminotransferase and Alanine Aminotransferase Activities) and An optimized lactate dehydrogenase release assay (explained by Kaja, et al. [55].

Statistical Analysis

Data were analysed using a commercially available statistics software package (SPSS Statistics for Windows, Version 20.0. IBM Corp., Armonk, NY, USA). All data were presented as the mean \pm SD for comparisons. Comparisons between groups were performed using the Kruskal Wallis analysis of variance for unpaired comparisons, followed by the Mann Whitney U test. The $P < 0.05$ was considered significant.

Results and Discussion

The results of the attempt on controlling the influence of biochemical changes induced by DMBA through the Methanolic Maceratives of Pre-pupal Stages of Black Soldier Fly, *Hermetia illucens* (L.) (MMPPSBSF) in rats are summarized in table (Table 1) and presented in Figures (Figures 1-4). The bioassay of total serum protein expedients measures the total protein contents of the blood. It also expedients the amounts of albumin and globulin, the two major groups of proteins. Albumin is synthesized mainly in the liver. Albumin helps keep the blood from leaking out of blood vessels. Albumin also helps to carry some medicines and other substances through the blood. The albumin is important for tissue growth and healing. The globulin fraction includes several serum proteins (Carrier proteins; Enzymes and immunoglobulins). Most of the globulins are synthesized in the liver. Other globulins belong to the immune system.

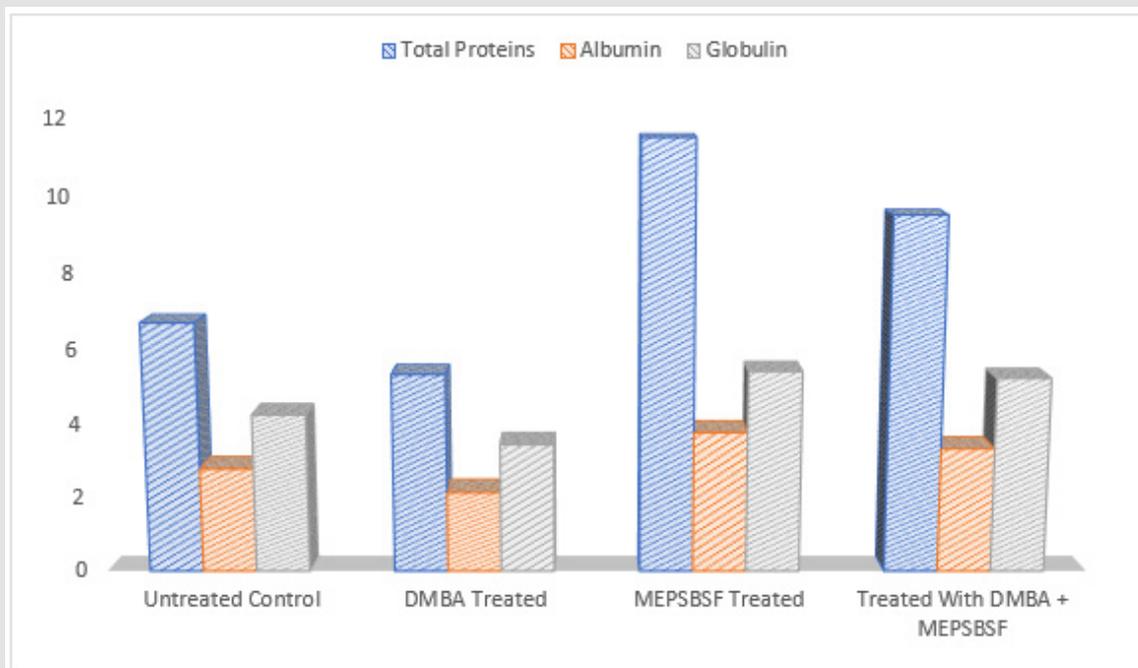


Figure 1: Influence of Methanolic Extractives of Preupal Stages of Black Soldier Fly (MEPSBSF) on level of Total Proteins, Albumin and Globulin in Serum in DMBA Induced Toxicity in Norwegian Rat, *Rattus norvegicus* (L).

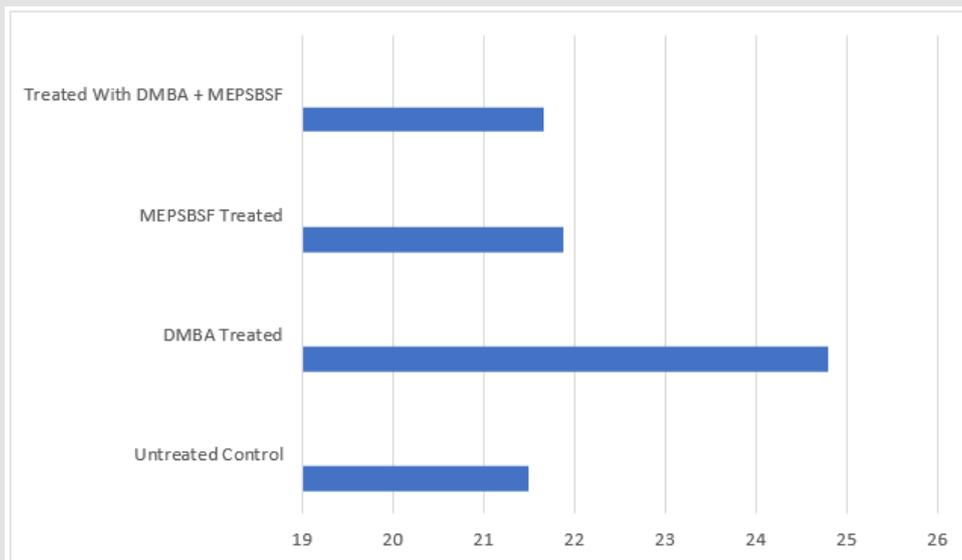


Figure 1: Influence of Methanolic Extractives of Prepupal Stages of Black Soldier Fly (MEPSBSF) on level of urea (mg/dL) in Serum in DMBA Induced Toxicity in Norwegian Rat, *Rattus norvegicus* (L).

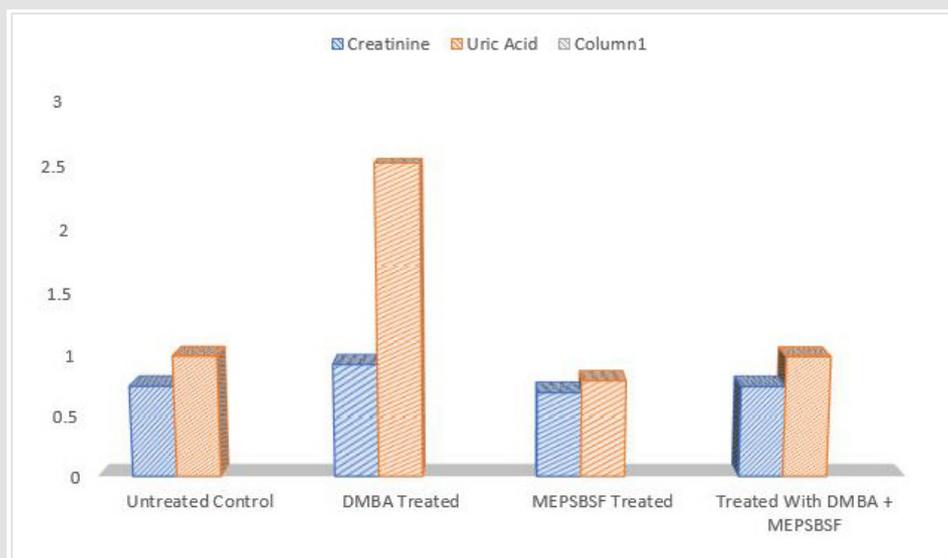


Figure 3: Influence of Methanolic Extractives of Prepupal Stages of Black Soldier Fly (MEPSBSF) on level of creatinine (mg/dL) and uric acid (mg/dL) in Serum in DMBA Induced Toxicity in Norwegian Rat, *Rattus norvegicus* (L).

The total protein level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of untreated control group was measured 06.715 (\pm 00.698) units (Table 1 & Figure 1). The total protein level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of DMBA treated group was measured 05.346 (\pm 00.984) units (Table 1 & Figure 1). There was 20.387 percent decrease in total protein contents of serum through the DMBA treatment. The total protein level in the serum of experimental animals (rat, *Rattus norvegicus*

L.) of MEPSBSF treated group was measured 11.527 (\pm 02.346) units (Table 1 & Figure 1). There was 71.660 percent increase in total protein contents of serum through the MEPSBSF treatment. The total protein level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of the group treated with DMBA followed by MEPSBSF was measured 09.536 (\pm 02.971) units (Table 1 & Figure 1). There was 42.010 percent increase in total protein contents of serum through treatment of DMBA + MEPSBSF.

Table 1: Effect of Methanolic Extractives of Prepupal Stages of Black Soldier Fly (MEPSBSF) on Biochemical Parameters of Serum in DMBA Induced Toxicity in Norwegian Rat, *Rattus norvegicus* (L).

Group Parameter	Untreated Control	DMBA Treated	MEPSBSF Treated	DMBA + MEPSBSF Treated
Total Protein (g/dL)	6.715	5.346	11.527	9.536
	(± 00.698)	(± 00.984)	(± 02.346)	(± 02.971)
	0	-20.387	71.66	42.01
Albumin (g/dL)	2.801	2.156	3.786	3.345
	(± 00.773)	(± 00.816)	(± 00.914)	(± 00.987)
	0	-23.027	35.166	19.421
Globulin (g/dL)	4.263	3.459	5.423	5.237
	(± 00.948)	(± 00.741)	(± 00.653)	(± 00.891)
	0	-18.859	27.21	22.847
Urea (mg/dL)	21.498	24.805	20.643	20.218
	(± 01.073)	(± 01.456)	(± 02.547)	(± 02.927)
	0	15.382	-3.977	-5.954
Creatinine (mg/dL)	0.741	0.918	0.689	0.739
	(± 00.023)	(± 00.079)	(± 00.237)	(± 00.308)
	0	28.571	-3.501	-0.269
Uric Acid (mg/dL)	0.987	2.513	0.786	0.982
	(± 00.034)	(± 00.493)	(± 00.018)	(± 00.111)
	0	154.6	-20.364	-0.506
AST (U/L)	114.93	237.37	117.68	118.76
	(± 06.145)	(± 41.459)	(± 23.346)	(± 26.686)
	0	106.53	2.392	3.332
ALT (U/L)	61.378	106.58	62.613	63.792
	(± 07.084)	(± 07.265)	(± 13.517)	(± 10.756)
	0	73.645	2.012	3.933
LDH (U/L)	153.78	257.65	156.37	164.31
	(± 14.354)	(± 32.137)	(± 34.759)	(± 39.186)
	0	67.544	1.684	6.847

Note: MEPSBSF: Methanolic Extractives of Prepupal Stages of Black Soldier Fly; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDH: Lactate Dehydrogenase. -Data is presented as Mean ± SD for n = 25. P < 0.05 SOD, "DMBA + MEPSBSF" compared with DMBA group. - P < 0.05 NO, "DMBA + MEPSBSF" compared with DMBA group. - P < 0.05 MPO, "DMBA + MEPSBSF" compared with DMBA group.

The albumin level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of untreated control group was measured 02.801 (± 00.773) units (Table 1 & Figure 1). The albumin level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of DMBA treated group was measured 02.156 (± 00.984) units (Table 1 & Figure 1). There was 23.027 percent decrease in albumin contents of serum through the DMBA treatment. The albumin level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of MEPSBSF treated group was measured 03.786 (± 00.914) units (Table 1 & Figure 1). There was 35.166 percent increase in albumin contents of serum through the MEPSBSF treatment. The albumin level in the serum of experimental animals (rat, *Rattus norvegicus*

L.) of the group treated with DMBA followed by MEPSBSF was measured 03.345 (± 00.987) units (Table 1 & Figure 1). There was 19.421 percent increase in albumin contents of serum through treatment of DMBA + MEPSBSF.

The globulin level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of untreated control group was measured 04.263 (± 00.948) units (Table 1 & Figure 1). The globulin level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of DMBA treated group was measured 03.459 (± 00.741) units (Table 1 & Figure 1). There was 18.859 percent decrease in globulin contents of serum through the DMBA treatment. The globulin level in the serum of experimental animals (rat, *Rattus norvegicus* L.)

of MEPSBSF treated group was measured 05.423 (\pm 00.653) units (Table 1 & Figure 1). There was 27.210 percent increase in globulin contents of serum through the MEPSBSF treatment. The globulin level in the serum of experimental animals (rat, *Rattus norvegicus* L.) of the group treated with DMBA followed by MEPSBSF was measured 05.237 (\pm 00.891) units (Table 1 & Figure 1). There was 22.847 percent increase in globulin contents of serum through treatment of DMBA + MEPSBSF.

Administration of DMBA caused decrease in levels of total proteins, albumin and globulin (Table 1; Figure 1). The decrease in their levels in plasma was reported in nephritic syndrome, inflammation, and chronic diseases etc [56,57] and ascribed to change in proteins synthesis and/or their metabolism. The DMBA treatment might have adversely affected the proteins synthesis and their metabolism. Treating the experimental animals (rat, *Rattus norvegicus* L.) with Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) in present attempt was found to cause a significant increase in the levels of total proteins, albumin and globulin in the serum. Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) thus, providing protection through maintenance of level of the total proteins, albumin and globulin in the serum.

DMBA Administration in the body of experimental animals (rat, *Rattus norvegicus* L.) in present attempt was found resulted in elevation of urea (15.382 percent), uric acid (154 percent) and

creatinine levels (28.571 percent) (Table 1, Figures 2 & 3). The elevation in the levels of urea, uric acid and creatinine in DMBA-treated group of experimental animals (rat, *Rattus norvegicus* L.) is considered as one of the markers of renal dysfunction [56,57]. Treating the experimental animals (rat, *Rattus norvegicus* L.) with Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) in present attempt was found to cause a significant decrease in the levels of urea, uric acid and creatinine in the serum (Table 1; Figures 2 & 3). This observation, therefore appears to have a potential ability of Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) to maintain renal function and avoid hypercatabolism in the DMBA-treated experimental animals (rat, *Rattus norvegicus* L.).

DMBA Administration in the body of experimental animals (rat, *Rattus norvegicus* L.) in present attempt was found resulted in elevation of the velocity of biochemical reactions catalysed by the enzyme Aspartate-Aminotransferase (AST); enzyme Alanine Aminotransferase (ALT) and enzyme Lactate dehydrogenase (LDH) (Table 1 and Figure 4). El-Demerdash, et al. [58-60] reported the increase in the activities of enzyme Aspartate-Aminotransferase (AST); enzyme Alanine Aminotransferase (ALT) and enzyme Lactate dehydrogenase (LDH) as a pathological change in the tissues like liver, kidneys heart and skeletal muscles. Treating the experimental animals (rat, *Rattus norvegicus* L.) with Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF) in present attempt was found to cause a significant protective influence.

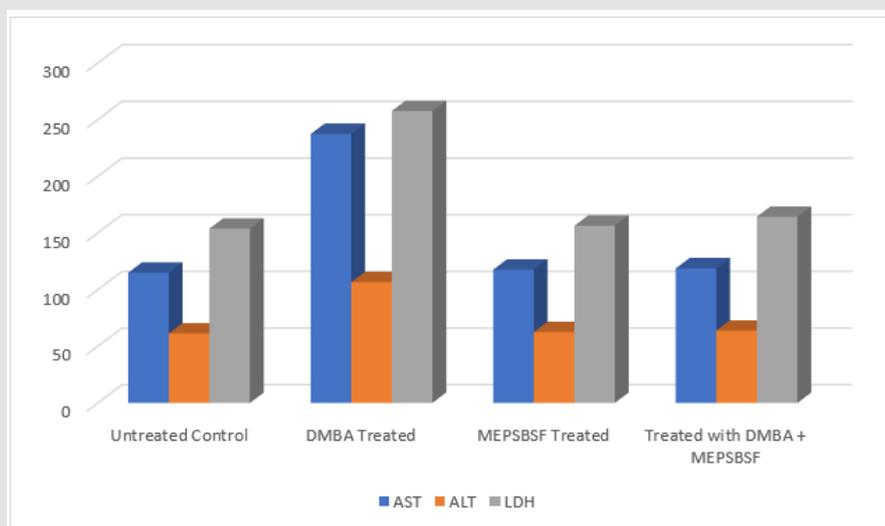


Figure 4: Influence of Methanolic Extractives of Prepupal Stages of Black Soldier Fly (MEPSBSF) on Activity of Aspartate-aminotransferase (AST) Alanine aminotransferase (ALT) and Lactate Dehydrogenase (LDH) in Serum in DMBA Induced Toxicity in Norwegian Rat, *Rattus norvegicus* (L).

Conclusion

In conclusion, the present attempt demonstrated that, the Methanol Extractives of Pre-pupal Stages of Black Soldier Fly

(MEPSBSF) could protect animals against detrimental influence induced by DMBA. The protective influence confirms the free radical scavenging ability of the Methanol Extractives of Pre-pupal Stages of Black Soldier Fly (MEPSBSF).

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