

# Mobile phone radiation induces sedation in *Periplaneta americana*

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**Exposure of adult male *Periplaneta americana* to electromagnetic radiation (EMR) emitted by mobile phones has resulted in sharp changes in the various enzymes systems of fat body and haematological profile. Sharp decline in the protein content of fat body together with increase of total free amino acids was observed. While the activity of glutamate oxaloacetate transaminase showed a significant decrease, that of glutamate pyruvate transaminase showed a sharp increase. The content of glucose and uric acid of fat body showed a sharp increase as well. A proteolytic enzyme, leucine amino peptidase showed significant decrease at the third hour of treatment, but a sharp increase at the sixth hour. Another proteolytic enzyme, cathepsin-D showed significant increase in activity both at third and sixth hour. Content of acetylcholine in the central nervous system showed a sharp increase, and organisms were found to be inert and lethargic after the third hour of EMR treatment. Total haemocyte count showed a sharp decline at the third hour, but a sharp increase at the sixth hour, together with imbalance and fluctuations on differential count. Cytopathological changes evidenced by lack of membrane integrity on plasma membrane and nuclear membrane, particularly on granulocyte were also observed. The present study revealed that continuous exposure to EMR of cell phones can result in widespread effects on the brain, neurons, developing cells and enzyme systems.**

**Keywords:** Electromagnetic radiation, mobile phone, *Periplaneta americana*, sedation.

MOBILE phones are now an integral part of modern life. Widespread use of mobile phones has been accompanied by the installation of increasing number of base station antennae on masts and buildings in both urban as well as rural areas. These base stations emit electromagnetic radiations (EMRs) at high frequencies ranging from 900 and 1800 MHz. Mobile phones and base station towers emit microwave radiation, which is a type of radio frequency radiation (RFR), a part of the spectrum of EM waves. EMR is a form of energy that is propagated through free space or through a material medium in the form of EM waves. EMR is emitted and absorbed by

charged particles<sup>1</sup>. Inhabitants living near mobile phone base stations are reported to suffer from headache, memory changes, dizziness, tremors, depressive symptoms and sleep disturbances<sup>2,3</sup>.

Radio waves of mobile phones can penetrate our brain and ear, and cause a decrease in cholinergic activity and altered gene expression in cerebellum<sup>4</sup> and alteration in cognitive functions<sup>5,6</sup>.

Dielectric heating by EMR through anthropogenic activity is now acting as a major physical stress to various types of organisms, including humans. Even though there are many reports on the adverse effect of EMR, there is not sufficient experimental evidence for these arguments. So we have conducted some basic studies on the effect of EMR emitted by mobile phones on *Periplaneta americana* as an experimental organism.

## Materials and methods

Adult male cockroaches were used as the experimental organism because of their large size and generalized morphology. Fat body of cockroaches was taken for various biochemical investigations. Haemolymph was also obtained to study the total haemocyte count (THC) and differential haemocyte count (DHC).

### *Treatment by electromagnetic radiation*

Two separate plastic containers of equal size (30 × 18 cm) were taken. Then 15 healthy male cockroaches were put in each box. The test box containing cockroaches was exposed to radiation from mobile phone for 1, 3 and 6 h by a full call (approximately 1 min) per 5 min to the mobile phone that was kept inside the box. A standard cell phone with a frequency band width of 900 MHz, power 2 W and specific absorption rate 0.35 was selected for radiating the specimens. The group of cockroaches inside the control box was also dissected out simultaneously and kept in ice-cold condition. Then 100 mg of fat body was weighed from each eppendorf tube. The amount of protein<sup>7</sup>; total free amino acids<sup>8</sup>, transaminases enzyme (GOT and GPT)<sup>9</sup>, glucose<sup>10</sup>, uric acid (standard kit manufactured by Biolabs), leucine amino peptidase<sup>11</sup>, and cathepsin-D<sup>12</sup> present in the tissue were estimated using standard protocols. Brain and

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central nerve cord of 10 cockroaches were dissected out (approximately 50 mg), and the content of acetylcholine and acetylcholinesterase were assayed<sup>13</sup>.

### Haematological studies

Haemolymph of cockroaches, both control and treated ones, was collected to study THC and DHC.

**Total haemocyte count:** Haemolymph was obtained by cutting the antenna and leg (coxa region) of the cockroach. The haemolymph that oozed out was collected in eppendorfs tube and diluted with Turk's fluid (20 times). THC was obtained using Neubauer haemocytometer. The number of circulating haemocytes per cubic millimetre ( $\text{ml}^3$ ) was calculated<sup>14</sup>.

**Differential haemocyte count:** A drop of haemolymph was placed on a clean microscopic glass slide and a thin smear was made by drawing the second slide across the first one at 45° angle. The smear was air-dried and stained using Giemsa stain for 20 min. The slide was then washed in running water to remove excess stain. The slide was then air-dried and viewed under high power of a compound microscope (Labomed with camera attachment, magnification = 400×) for identification of the cells. Individual slides corresponding to each treated and untreated samples were then prepared and the cells were counted. The haemocytes were identified by their distinguishing characters<sup>15,16</sup>. The percentage of different types of haemocytes in both treated and control samples was calculated.

### SDS page

For this, 100 mg fat body was homogenized in ice-cold phosphate buffer (1 ml, pH 7.2), centrifuged in cold centrifuge at 8000 g for 15 min. Then SDS PAGE was carried out with the supernatant as described by Laemmli<sup>17</sup> and the electropherogram was subjected to Gel-Doc analysis.

### Statistical analysis

Statistical analysis was done using IBM SPSS statistics 20 software.

## Results

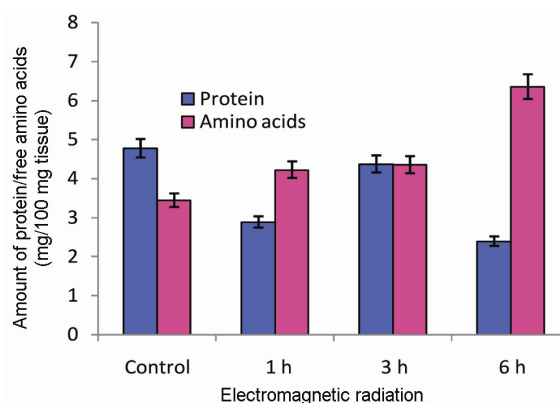
The treatment of *P. americana* with EMR for three durations (1, 3 and 6 h) resulted in a decline in protein content of fat body when compared to control (Figure 1). The content of total free amino acids on the other hand showed an increase under identical conditions (Figure 1). During EMR treatment, the activity of glutamate oxaloacetate transaminase (AsAT/GOT) showed a slight

but significant decrease when compared to control (Figure 2), but the activity of glutamate pyruvate transaminase (AIAT/GPT) showed a slight increase. In normal healthy *P. americana*, the ratio of GPT to GOT is 0.04, which increased to 0.10 at the third hour and reached up to 0.40 at the sixth hour of EMR treatment (Figure 3).

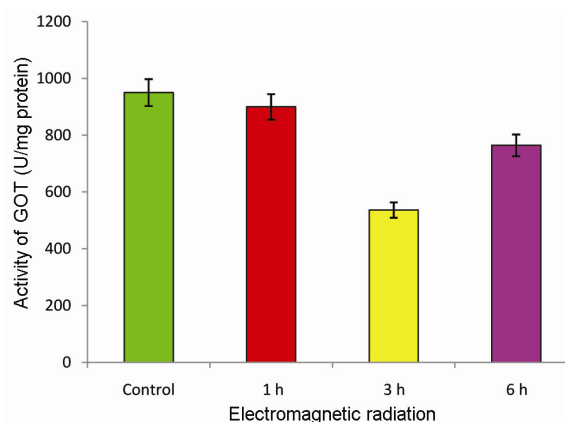
On treatment with radiation for 1 and 3 h, *P. americana* showed a decline in glucose content of fat body when compared to control. However, at the sixth hour of EMR treatment, glucose content showed a sharp increase. Similar results were obtained for the content of uric acid of fat body (Figure 4).

The activity of leucine amino peptidase (LAP) showed a decline during the initial hours of EMR exposure. When the cockroach was exposed to radiation for 6 h, the activity of LAP increased sharply (Figure 5). Cathepsin-D showed a gradual increase in activity in a dose-dependent manner (Figure 5).

Acetylcholine content of the central nervous system showed significant increase during the EMR treatment



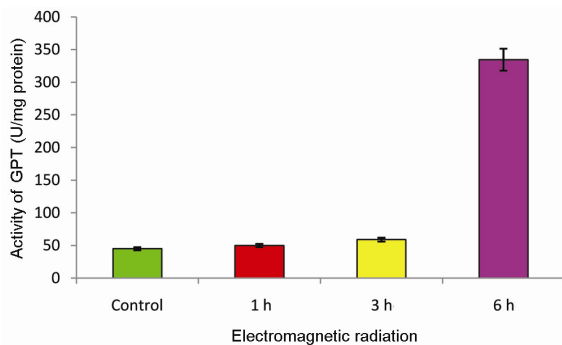
**Figure 1.** Effect of EMR on protein content and total free amino acids of fat body. All values are mean  $\pm$  SEM,  $n = 6$ . Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.



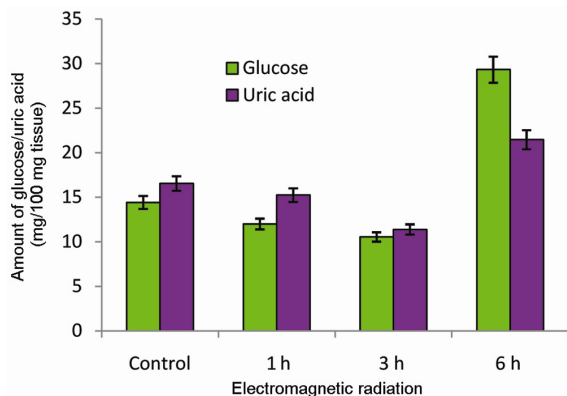
**Figure 2.** Effect of EMR on the activity of glutamate oxaloacetate transaminase. All values are mean  $\pm$  SEM,  $n = 6$ . Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.

(Figure 6), but the activity of acetylcholinesterase showed a decrease (Figure 7).

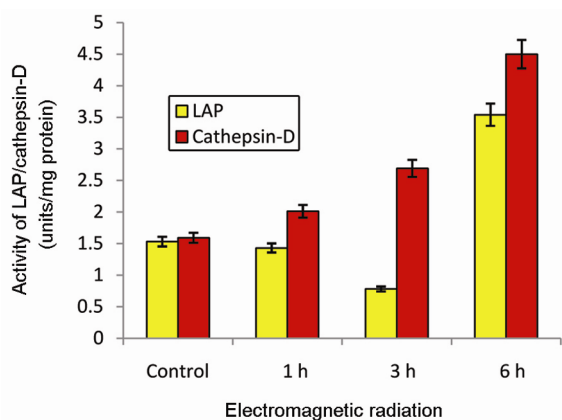
Exposure to EMR caused significant changes in THC and DHC. It also caused cytopathological changes in the haemocytes. Six well-defined haemocyte types were identified in the haemolymph of both treated and untreated cockroaches. These include: prohaemocytes,



**Figure 3.** Effect of EMR on the activity of glutamate pyruvate transaminase. All values are mean  $\pm$  SEM,  $n = 6$ . Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.



**Figure 4.** Effect of EMR on the content of glucose and uric acid of fat body. All values are mean  $\pm$  SEM,  $n = 6$ . Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.

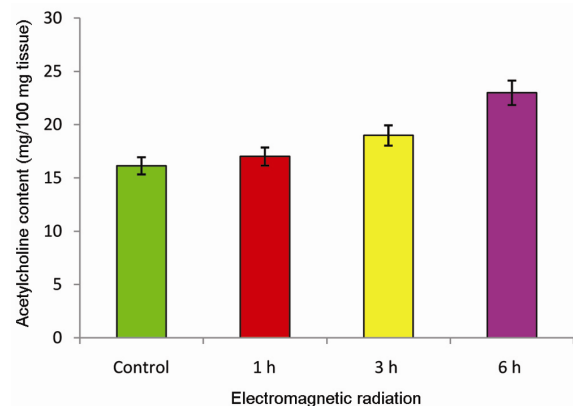


**Figure 5.** Effect of EMR on the activity of leucine amino peptidase and cathepsin-D of fat body. All values are mean  $\pm$  SEM,  $n = 6$ . Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.

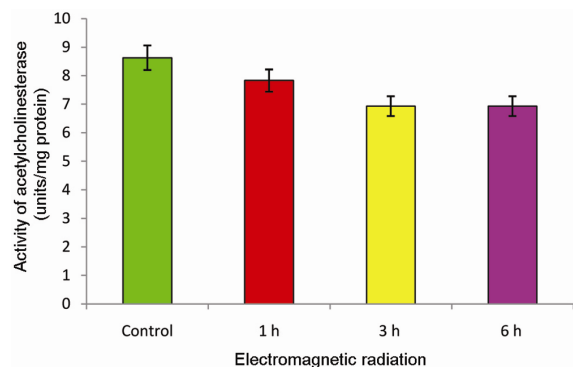
plasmatocytes, granulocytes, adipohaemocytes, oenocytoids and spherulocytes. DHC of healthy cockroaches showed that 60% of the total haemocyte population included granulocytes and spherulocytes; among them 28.24% were granulocytes and 29.9% were spherulocytes, indicating their equal share in haemolymph. The prohaemocytes and plasmatocytes formed about 18.8% and 16.2% respectively. The remaining haemocyte types are present in the circulation, but their proportion is not greater than 6.0%.

Treatment of *P. americana* with EMR from mobile phone for 1 h (just 12 calls) caused a sharp decrease in population of spherulocytes to approximately 5%, with simultaneous elevation of granulocytes to 53.09% (Figure 8). The population of prohaemocytes also increased from 18.8% to 26.55%, and the proportion of spherulocytes decreased from 29.91% to 5.31%. Similar results were obtained for plasmatocytes; their proportion decreased from 16.2% to 6.19%. The proportion of oenocytoid showed a slight change.

Treatment of *P. americana* with EMR for 3 h resulted in further increase of granulocytes to 58.34% of total haemocytes and oenocytoids to only 5.55% of haemocytes. The share of spherulocytes diminished from



**Figure 6.** Effect of EMR on the acetylcholine content in the central nervous system. All values are mean  $\pm$  SEM,  $n = 6$ . Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.



**Figure 7.** Effect of EMR on the activity of acetylcholinesterase in the central nervous system. All values are mean  $\pm$  SEM,  $n = 6$ . Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.

29.91% to 6.48%. Similar results were obtained for plasmatocytes. The proportion of prohaemocytes mostly remained unchanged during EMR treatment, with an insignificant change from 18.8% to 20.37%.

However exposure to EMR for 6 h showed that the proportion of granulocytes increased significantly from 28.24% to 62.73%. The proportion of spherulocytes decreased to 11.82%. The number of prohaemocytes did not show any significant difference in both control and treated cockroaches. The proportion of adipohaemocytes was never above 6.0% in both treated and untreated cockroaches. Increased proportion of granulocytes was observed after exposure to EMR (Figure 8).

THC also showed significant variation on treatment with EMR. For normal healthy cockroaches, it was 7750/mm<sup>3</sup>. When the cockroaches were treated with radiation for 1 h the number decreased from 7750/mm<sup>3</sup> to 6580/mm<sup>3</sup>, while on exposure to radiation for 3 h the number further decreased to 4500/mm<sup>3</sup>; however, at the sixth hour of EMR treatment, it increased significantly to 11,200/mm<sup>3</sup> (Table 1).

Exposure of *P. americana* to EMR also resulted in some cytopathological changes in the three populations of haemocytes, i.e. granulocytes, spherulocytes and oenocytoids. Rupture of cell membrane was observed in 50% of

granulocytes at the sixth hour of treatment but in spherulocytes lack of cell membrane integrity was observed in more than 75% of the population (Figure 9 g and h). However, in some oenocytoids the nuclear membrane ruptured, but the cell membrane was intact (Figure 9 i).

*P. americana* showed drowsiness after the third hour of EMR treatment. They did not even move their antennae after the treatment.

### Protein profile

SDS-PAGE electrophoresis of fat body protein of *P. americana* in normal state and under stress condition revealed that EMR treatment resulted in the appearance of additional bands together with disappearance of some protein bands during treatment. There were only nine bands in control cockroaches, but 11 bands were observed in cockroaches treated with EMR for 1 h. The number of protein bands was only 9 in 3 h of treatment, which again increased to 10 bands in 6 h of exposure (Figure 10). The above details will be clear from Gel-Doc analysis data (Table 2).

### Discussion

Mobile phones have now become a necessary tool in our daily lives, enabling us to communicate easily with others. Cell phone usage is a major public health concern because of potential risk of chronic exposure to low level of radio frequency and microwave radiation that in close proximity to the body<sup>3</sup>.

Just a few calls at a duration of 1 min at every 5 min was sufficient for *P. americana* to undergo drastic changes in the metabolism of protein characterized by depletion of fat body protein accompanied by a sharp increase of total free amino acids. A similar result was observed in another study on the red flour beetle (*Tribolium castaneum*) exposed to low intensity microwave radiation (LIMR; ≤ 2.0 kW/kg), where protein content decreased while the total free amino acids content increased<sup>18</sup>.

Insects are characterized by the presence of very high content of free amino acids<sup>19</sup>. Being a typical insect, fat body of *P. americana* showed very high content of free amino acids. Free amino acids are important in insects because of their role as energy releasers<sup>20</sup>, acting as agents maintaining acid–base balance, as precursors of secondary metabolism and also in detoxification mechanism. Free amino acids have a major role in maintaining the pH of haemolymph, and hence elevation of free amino acids will alter the pH of body fluids<sup>21</sup>. In humans certain situations such as chronic infections, electric shock and in post-operative conditions decrease the protein content together with increase in free amino acids<sup>22</sup>. The treatment of insects with insecticides also resulted in decline in protein content with subsequent increase of free amino acids.

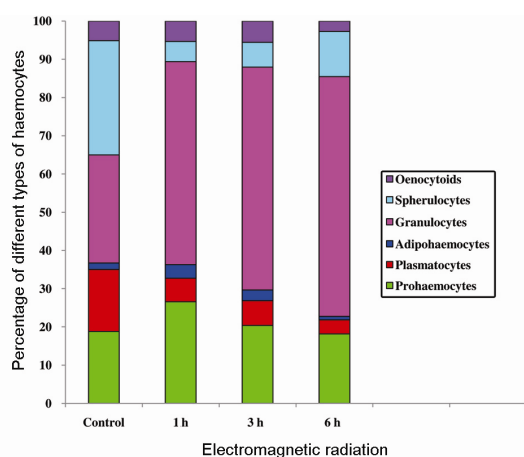
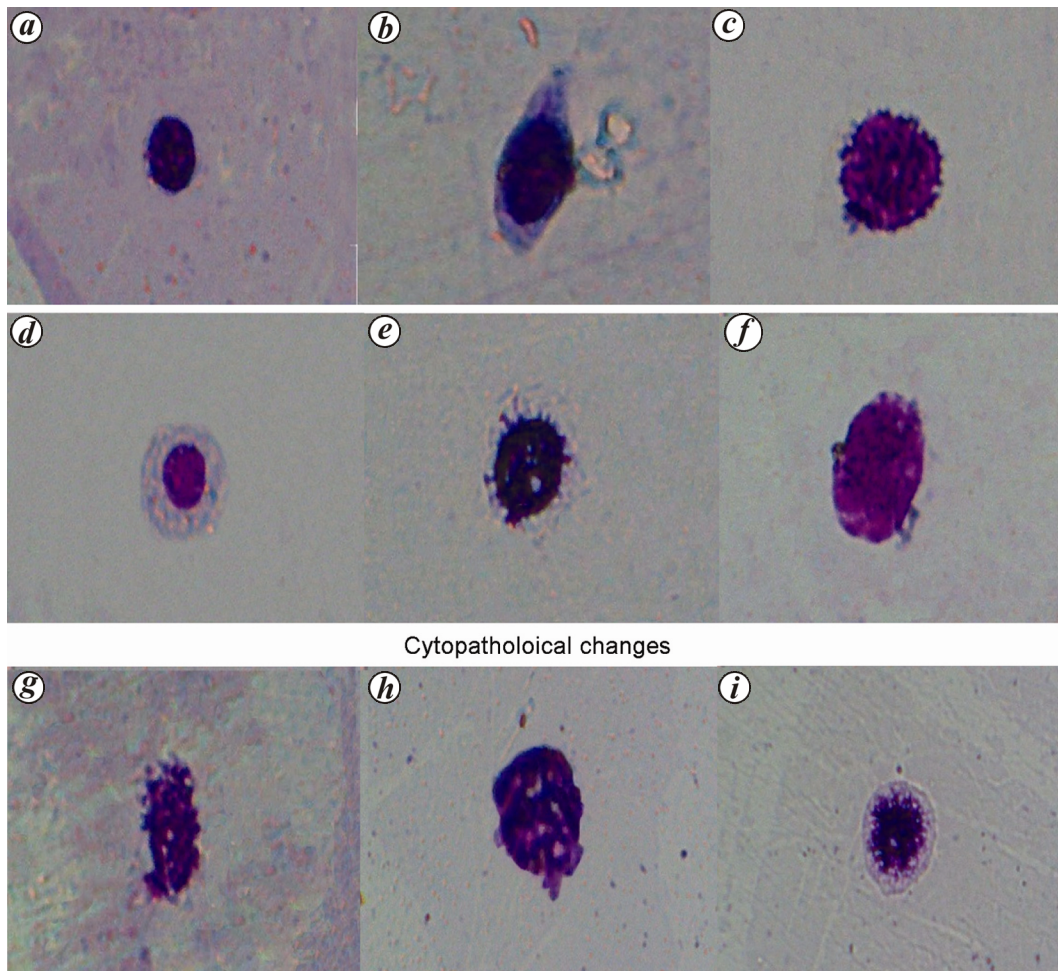


Figure 8. Effect of EMR on the differential haemocyte count.

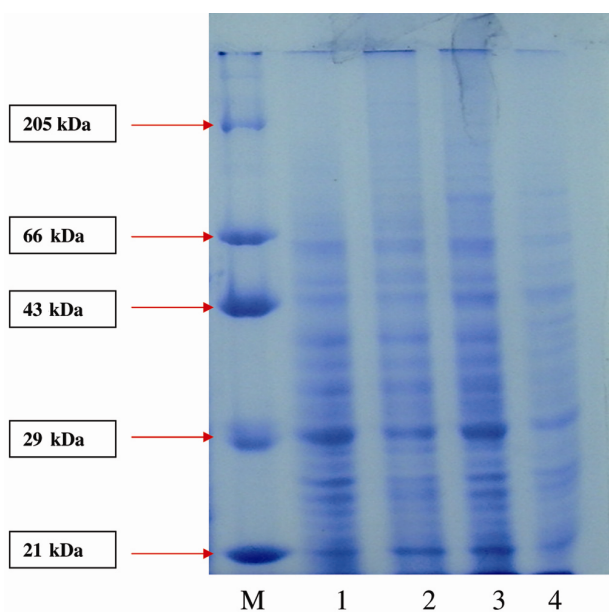
Table 1. Effect of cell phone radiation on total haemocyte count

Cell phone radiation	Total haemocyte count
Control	7,750 ± 80
1 h	6,580 ± 67
3 h	4,500 ± 52
6 h	11,200 ± 97

Values are mean ± SEM, and are expressed as number/cubic millilitre. Values are significantly different,  $P \leq 0.05$  with respect to control.



**Figure 9.** Cytopathological changes induced by EMR on the haemocytes. *a*, Prohaemocyte; *b*, Plasmatocyte; *c*, Granulocyte; *d*, Oenocytoid; *e*, Adipohaemocyte; *f*, Spherulocyte; *g*, Granulocyte, *h*, Spherulocyte; *i*, Oenocytoid.



**Figure 10.** Electropherogram showing protein profile of *Periplaneta americana* after exposure to cell phone radiation. Lane M, Molecular marker; line 1, 6th hour; lane 2, 3rd hour; lane 3, 1st hour; lane 4, Control.

Exposure of *P. americana* to EMR resulted in imbalance in the functioning of two marker enzymes of amino acid metabolism, i.e. aspartate amino transferase and alanine amino transferase. They are marker enzymes of liver function in humans<sup>22</sup>. In normal insects these two enzymes are 50 to 75 times more active than in human blood, which indicates that the rapid turnover of amino acid to keto acid and keto acid to amino acid enzyme might play a major role in energy metabolism of insects because they possess free amino acids levels several times higher than the mammalian system. So exposure of insects to EMR might affect energy metabolism in their body through imbalance in the activity of transaminase enzyme system. Response of the above two transaminases was different under EMR exposures. A shift in the ratio of GPT to GOT from 0.04 to 0.40 was a clear indication of the disruption of amino acid metabolism in insects exposed to EMR. In humans, elevation of both amino transferases is an indication of hepatitis<sup>23</sup>.

In the present study we observed increase in glucose and uric acid in fat body of *P. americana* under prolonged exposure to EMR. The normal content of glucose

**Table 2.** Gel-Doc analysis of electrophorogram of *Periplaneta americana* after exposure to cell phone radiation

Band	Molecular weight of protein bands (kDa)			
	Normal control	Duration of EMR treatment (h)		
		6	3	1
1	96.42	70.15	71.02	93.71
2	75.19	57.92	58.87	72.77
3	54.48	52.73	52.95	59.11
4	46.09	41.12	41.96	53.16
5	38.68	36.09	36.68	42.31
6	26.36	31.16	32.06	36.83
7	19.73	24	24.6	32.32
8	18.34	18.49	18.79	24.7
9	12.86	16.83	12.5	19.02
10	Not seen	12.35	Not seen	17.25
11	Not seen	Not seen	Not seen	12.65

Rf values, band area and band volume are not included in the table.

in mammals is between 80 and 120 mg/100 ml blood. However, in the insect system such as haemolymph of *Oryctes rhinoceros*, the content of glucose was well below that of the mammalian system<sup>24</sup>. In fat body of *P. americana*, the normal content of glucose was only 15/100 mg tissue. This indicated that glucose has no significant role as an immediate energy releaser in the insect system, and in most cases keto acids derived from amino acids may act as energy sources. The present study revealed that the content of uric acid, the chief nitrogenous waste material of insects, showed a significant decrease in its content at the third hour of exposure to both stressors. This decrease may be due to initial dormancy of the organism under stress condition. When the stress condition was prolonged to 6 h, the body may have shifted to increased catabolism of amino acids as a life-saving mechanism. This may be the reason for increase of uric acid in fat body.

The efficiency of LAP in the immune system has been well documented, rendering protection in animals when exposed to radiation<sup>25</sup>. LAP showed elevated activity in larval insects than in adults<sup>26</sup>. In the present study activity of LAP declined up to the third hour and at the sixth hour of EMR treatment, it rose sharply. This is detrimental to the existence of the organism.

Activity of cathepsin-D, as enzyme is involved in tail regression during the metamorphosis of amphibian tadpoles, is an important enzyme which regulates turnover of protein to amino acids. In the present study, this enzyme showed a sharp increase in its activity after exposure to EMR. In the larvae of *Culex quinquefasciatus*, activity of cathepsin-D showed a sharp increase when they were exposed to insecticides, which also resulted in increase of free amino acids in the haemolymph to several fold<sup>19</sup>.

Acetylcholine is an organic, polyatomic ion that acts as a neurotransmitter in both the peripheral nervous system and central nervous systems in many organisms, includ-

ing humans. EMR treatment resulted in gradual elevation of acetylcholine in central nervous system, coupled with decrease in the activity of acetylcholinesterase. During neurotransmission, acetylcholine is released from the nerve into the synaptic cleft and binds to acetylcholine receptors on the post-synaptic membrane, relaying the signal from the nerve. Acetylcholine esterase, also located on the post-synaptic membrane, terminates the signal transmission by hydrolysing acetylcholine. Most insecticides of group-chlorinated hydrocarbons and organophosphorous compounds are well-known inhibitors of acetylcholinesterase; they cause paralysis and death of insects through accumulation of acetylcholine at the axon terminals. In the present study, the observed inertia and lethargic nature of cockroaches after treatment by EMR may be due to accumulation of acetylcholine. In the larvae of *C. quinquefasciatus*; toxicity by chlorinated hydrocarbon and organophosphorus compounds resulted in paralysis and accumulation of acetylcholine<sup>27</sup>. So EMR might have caused toxicity equivalent to insecticides on the nervous system.

Exposure of *P. americana* to EMR resulted in the imbalance in differential count of haemocytes. A drastic change in the number of haemocytes was observed in all populations of cells, but the granulocyte population was most affected. This clearly indicated that the structure of haemolymph was badly affected by EMR. In *Drosophila melanogaster*, it was observed that EMR exposure badly affected the reproductive efficiency of the flies<sup>28</sup>. Studies on ants<sup>29</sup> and honey bees<sup>30</sup> have shown that GSM (global system for mobile communication) has adverse effects on them.

According to Sainudheen<sup>31,32</sup>, EMR is crippling the navigation skills of honey bees and worker bees are unable to return to their nests. This phenomenon has given rise to several problems in bee-keeping industry and hives in the vicinity of base station towers have been

deserted without any known cause. Establishment of base station antennae has resulted in the collapse of honey-bee colonies in surrounding areas. This phenomenon is called 'colony collapse syndrome'. The present study has provided evidence on the physiological basis of colony collapse syndrome. In the United States, certain strict rules exist which prevent establishment of base stations in populated areas. According to the US regulations, a particular area located at 200 m diameter around a base station tower is called the blue zone, which is avoided by most insects and birds. The present study clearly explained the physiological and biochemical basis of adverse effect of EMR and is a warning for the judicious use of mobile phones.

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